The Physiological Response of sub-Arctic Lichens to Their Abiotic Environment

HENRI, DOMINIC,CHARLES

The Physiological Response of sub-Arctic Lichens to Their Abiotic Environment

Abstract

The effect of environmental factors on metabolic processes of sample thalli, of four sub-Arctic lichen species from Abisko, Sweden (68.37˚N, 18.69˚E). *Nephroma arcticum*, *Cladina mitis*, *C. stellaris* and *C. rangiferina*, which are found in contrasting niche habitats within the same ecosystem, have been studied. An oxygen electrode apparatus has been used to study the effects of air temperature, thallus irradiance intensity and climatic regime during thallus storage, on the rate of net photosynthesis and dark respiration of sample thalli, in a series of studies on the four lichen species named above.

Environmental temperature and light intensity both significantly affected the rate of thallus net photosynthesis (NP) in samples of all four species. The mean rate of thallus NP was significantly depressed at 17^{o}C in all four species due to an exponential increase in respiratory rate with rising temperature. Maximal NP was achieved at 5^{o}C in *N. arcticum*, 10^{o}C in *C. rangiferina*, and NP at 5 & 10^{o}C were not significantly different in *C. mitis* and *C. stellaris*. Optimum light intensity for photosynthesis was 340 µmol m^{-2} s^{-1} in all four species; however, temperature significantly increased the light compensation point of photosynthesis. Furthermore, mean light compensation points were greater in the more shaded adapted species *N. arcticum* and *C. mitis*; suggesting, as found in vascular plants, lichens exhibit photosynthetic characteristics adapted to their abiotic environment. *C. mitis* thalli did not provide any evidence of plastic adaptation to temperature over a six day storage period; whereas, *N. arcticum* samples exhibited increased NP after six days at 10 & 15^{o}C compared to non-acclimatized samples. Prolonged storage at 10^{o}C appeared to be detrimental to samples of *C. stellaris*. Responses to temperature and light intensity were similar to those found, in similar species, in photosynthetic rate investigations utilizing IRGA apparatus; validating the use of O_2 electrode experiments using small samples of thallus tissue, as used in this investigation.

The response of species, within this study, to environmental factors was slightly different to those obtained in similar studies for North American populations, which suggests that lichens may exhibit some within-species genetic variation between lichen populations from different continents. Analysis of moisture availability, from past climate-data of the region from which the lichen populations are found, suggests that the growth season of sub-Arctic lichens is very different to that of vascular plants that are within the same ecosystem. Future warming may cause longer, wetter Arctic Autumns and Springs, which would benefit lichen biomass, but environmental warming would suppress lichen growth and promote vascular plant productivity.

The Physiological Response of sub-Arctic Lichens to Their Abiotic Environment

A report of investigations undertaken to study the effect of the external environment, on photosynthesis and respiration in lichen species, which are common in tundra-heath ecosystems from Abisko, Sweden.

Dominic Charles Henri

Supervisors: Dr. Robert Baxter and Prof. Brian Huntley

Durham University, Department of Biological and Biomedical Sciences

Thesis for the degree of MSc Biology by research

October 2010.

Table of Contents

List of Illustrations

1. Introduction

Figures

Tables

2. Samples

Figures

Tables

3. Methodological development

Figures

4. The effect of short-term exposure, to varied air temperature, on the light response of photosynthesis and dark respiration, of four species of lichens: *N. arcticum*, *C. mitis*, *C. stellaris* and *C. rangiferina*

Figures

Tables

5. The effect of long-term temperature regime on the photosynthetic rate of *N. arcticum*, *C. stellaris* and *C. mitis* thalli

Figures

Tables

6. Discussion of results obtained over the course of the study period

Figures

Tables

Appendix 1

Figures

Acknowledgements

Many thanks to Dr Robert Baxter, without whom this work would not have been possible, it has been a pleasure. Also thanks to Dr Steve Hancock who was of great aid in the data compilation and manipulation tasks. To the entire ecology department, the inhabitants of which create the most pleasant working environment I have ever encountered. Finally thank you to Laura Wilson for your hard work and to the University Hardship Fund committee for giving me the financial backing to complete my degree.

Dedication

This script is dedicated to my family for all their support, both emotional and fiscal, and allowing me to follow through on my ambitions. It is dedicated to my friends at Durham University (although they may never read this) who have made my four years at Durham more of a holiday than a degree. It is also dedicated to anyone at university who is working to broaden their horizons and is struggling; it can be done and it is worth it. Finally but far from lastly, this is dedicated to Louisa Briggs, it could never have been finished without you Lou, you are the rule by which I have gritted my teeth and kept on working even, though it was all going wrong. I could never have worked as hard as I did without you beside me.

"Virtutis Fortuna Comes"

Chapter 1

Introduction

1.1 What are lichens?

A lichen can be defined as "*an association between a fungus, usually an ascomycete but in a few cases a basidiomycete or deuteromycete, and one or more photosynthetic partners, generally green algae or cyanobacteria. In all lichens the fungus forms a thallus or lichenized stroma that may contain unique secondary compounds.*" - (Ahmadjian, 1993).

Lichen thalli are composed of two organisms found together in a mutual-symbiotic relationship: a lichen-forming fungus (mycobiont) and a photobiont. The photobiont may be one or more green alga species, one or more cyanobacteria species or any combination of both. A lichen thallus is formed predominantly of fungal hyphae (~95%) (Baron, 1999). Within a thallus the mycobiont's hyphae exude chemicals which increase the permeability of the photobiont cell wall or cell membrane and allow increased transference of organic carbon from the photobiont to the mycobiont (Baron, 1999). The transference of organic carbon, produced through photosynthesis by the photobiont, satisfies the nutritional requirements of the mycobiont partner. Lichen-forming fungi are, in natural settings, obligate symboitic organisms; although they can be cultured separately from their photobiont symbiot *ex situ* (Honegger, 1998). As the photobiont is, more often than not, capable of survival without a fungal partner, and it has been shown that photobiont species grow at a slower rate than found outside of the lichen thalli, some consider the photobiont is being parasitised by the fungus (Ahmadjian, 1993). However Honegger (1998) suggests that the symbiosis allows many photobiont species to better disperse into areas where they could not otherwise survive (due to the considerable protective abilities of the fungal part of the thallus). Honegger (1998) also suggests that members of the algal genus *Trebouxia*, one of the most common green algal photobionts, are poor competitors in the natural world and gain a competitive advantage being associated with a mycobiont partner. More recent studies have shown that lichenised fungi produce a compound that diminishes damage in photobiont photosystems, from reactive oxygen species, during desiccation (Kosugi *et al.*, 2009). Furthermore photobionts which are part of a symbiosis are also known to maintain greater levels of photosynthesis, during adverse conditions, than isolated individuals of the same species (Brock, 1975). These benefits contradict the parasitic view of the lichen relationship, although it is probable that different lichen species have relationships ranging from symbiotic to parasitic (Honegger, 1998).

Past estimates of lichen-forming fungal species range between 13,500 and 18,000 (Hawksworth *et al.*, 1995; Sipman and Aptroot, 2001). According to Honegger (1998) lichen-forming fungi comprise ~21% of all known species of fungi. As the integration of the mycobiont and the photobiont is so intimate lichens have been considered to be a single organism; therefore a lichen species classification is denoted by a single binomial. This binomial is the name of the mycobiont partner as there are only 26-40 genera of photobiont found in lichen thalli, and many different fungi may share the same photobiont species (Baron, 1999). For example 85% of green photobionts are members of one of three algal genera (Baron, 1999). Also, lichen- forming fungi may form morphologically-identical thalli incorporating multiple algal species and can be considered the same species when found with either (or many) of the potential photobiont partners (Ihda *et al.*, 1993; Nash, 1996).

Although lichen-forming fungi have been originally classified under a single taxon, the Lichenes, it is now generally accepted that lichens are polyphyletic (Baron, 1999; Hibbett *et al.*, 2007; Honegger, 1998; Nash, 1996). The vast majority of lichen-forming fungi are found in the phylum Ascomycota; 95% according to Baron (1999) and 98% according to Lutzoni *et al.* (2001). Within the Ascomycota lichen-forming fungi are found in 13 orders (Hawksworth *et al.*, 1995). Lichen-forming fungi are also represented in the Basidiomycota. It has even been hypothesised that secondary loss of lichenisation has led to the evolution of major fungal lineages within the Ascomycota (Lutzoni *et al*., 2001). The evolutionary lineage of lichen-forming fungi is important as lichens exhibit a remarkable physical and ecological diversity which is an important consideration when drawing parallels between different lichen species.

1.1.1 Definitions

In this introductory review the physical response of lichens refers to the any change in thallus morphology or metabolic rate of the thallus bionts, for individuals of a species, to changing climatic factors (such as temperature). The ecological response corresponds to any change in the distribution and biomass of species as a whole. The focus of this study is on the Arctic in particular as it is a region in which lichen play an important role within a wide array of ecosystems. It is a region in which 11% of lichen species are endemic and over 50% of all the 'plant' species in the region are cryptogams (Callaghan *et al.*, 2004b). Lichen ecology is greatly undervalued, they are considered to be one of the most sensitive groups to climate change, and they mainly inhabit regions which are supposedly most vulnerable to future climate change (Callaghan *et al.*, 2004a; Callaghan *et al.*, 2004c; Serreze *et al.*,

2000). Yet as of 2009 only two species (0.01% of all lichen-forming fungal species described) had been evaluated by the ICUN (International Union for Conservation of Nature)**;** both of which are classified as threatened
(http://www.iucnredlist.org/documents/summarystatistics/2009RL_Stats_Table_1.pdf).

1.1.2 The physiology of lichens

Lichens are extremely hardy organisms and are dominant in some of the most inhospitable ecosystems on the planet (Nash, 1996). Their hardiness is of particular importance to astrobiologists as lichen thalli are capable of metabolic recovery after exposure to the vacuum and cosmic radiation of space; conditions which are inhospitable to even extremophilic bacteria (Sancho *et al.*, 2008). The nutrition of lichen thalli is not dependant on the mineral component of the substrate upon which they grow and hence lichen species are capable of colonising areas without soil, making them important components of primary succession (Kappen, 2000). Laboratory experiments have shown that lichens are capable of recovering full metabolic activity even after the formation of intra-cellular ice and desiccated lichen specimens have been shown to tolerate temperatures as low as -196˚C (Bjerke, 2009; Kappen, 2000).

Lichens have been shown to be capable of net positive photosynthesis (i.e. carbon uptake) at sub-zero temperatures and even under snow cover, in both *in-* and *ex-situ* experiments (Hajek *et al.*, 2001; Kappen *et al.*, 1995; Reiter *et al.*, 2008; Schroeter *et al.*, 1996; Schroeter and Scheidegger, 1995). Unlike vascular plants, lichens are poikilohydric, i.e. their cellular water content is dependent on the moisture available in the atmosphere.

Lichens are commonly subject to desiccation in their natural habitats; although there is variation in the rate of water loss between species (Baron, 1999). Consequently, lichens are considered to be desiccation-tolerant; i.e. they are capable of full metabolic recovery after the loss of 80-90% of protoplasmic water (Oliver *et al.*, 2000). This is a trait which is very rare in tracheophyte lineages: being found in only 60 angiosperm species, 60-70 pteridophyte species and no gymnosperm species (Oliver *et al.*, 2000). Desiccation-tolerance is a general trait found in lichen species regardless of lineage, but the speed and intensity of desiccation which can be tolerated differs between species. The degree of desiccation-tolerance shown by a thallus appears to be related to the ecology of the species; i.e. species that are more often subject to periods of desiccation will exhibit less damage and recover faster from desiccation (Beckett, 1995).

The extremophilic characteristics of lichen species mean they make up a major proportion of the biomass in regions where tracheophytes cannot establish or where they store the majority of their biomass underground (Shaver and Chapin, 1991). As such, lichens are important parts of Arctic, Alpine, Antarctic and some desert ecosystems (Kranner *et al.*, 2008). For example in Siberian tundra-heath regions lichens cover over 70% of the soil surface and in Arctic birch forest ecosystems areas of greater than 50% lichen cover are common, with ~1000km^2 of such area in the Norwegian region of Kautokeino alone (Biasi *et al.*, 2008; Tommervik *et al.*, 2009). Cryptogamic (i.e. lichen and moss) organisms are known to become increasingly important components of ecosystems with increasing latitude and altitude (Reiter *et al.*, 2008).

1.1.3 The importance of lichens in Arctic regions

The Arctic region is considered to be a considerable global carbon sink (Ping *et al.*, 2008). Global warming is predicted to change the Arctic climate in such a way that this will change the region into a significant carbon source (Biasi *et al.*, 2008). The carbon (C) balance of a region is regulated through two main processes: C-release which occurs mainly through heterotrophic soil respiration and C-accumulation which results through the conversion of atmospheric C into organic C by photosynthesis (Campioli *et al.*, 2009). Although lichen photosynthetic rate is considered to be much lower than vascular plants or mosses in the same region they may still comprise a significant component of the carbon cycle in Arctic regions (Biasi *et al.*, 2008; Uchida *et al.*, 2006). In the late summer, in Northern Swedish tundra (Abisko), 25% of all C accumulation, by photosynthetic organisms, is incorporated by non-vascular 'plants'. Understanding the response of lichen species to future climate change predictions may be an important factor in counteracting the potentially large addition of pre-historic carbon into the atmosphere.

Arctic ecosystems are generally considered to be limited by nitrogen (N) availability (Vitousek and Howarth, 1991) and it has been hypothesised that this is an important part of the success of lichens in Arctic ecosystems (for a detailed review of the subject see Cornelissen *et al.* (2007)). Lichens are an important part of the N-cycle in many habitats: cyanobacterial photobionts are often capable of fixing atmospheric N; some epiphytic lichens convert ammonium, from wet deposition, into nitrates or other organic nitrogen compounds which are more easily usable by tracheophytes (Oyarzun *et al.*, 2004). The mycobiont hyphae of saxicolous (species which grow on rocky substrata) lichen species have been shown to be an important part of the weathering of rocks and the release of compounds into the nutrient cycle (Adamo and Violante, 2000). The removal of epiphytic lichen, from

forests of *Quercus douglasii*, significantly decreased the rate of deposition of both N and phosphorus as lichen thalli act as a 'interception surface', by which nutrients, carried within the rain, are trapped in the canopy (Knops *et al.*, 1996). The nutrient cycling properties of lichen are an important part of the plant succession process in many ecosystems. Vitousek (1994) has shown that lichen species are one of the major components in the mechanism by which N is incorporated into soils on volcanic islands. Some cyanobacterial photobionts fix atmospheric N and N in rain is incorporated into a lichen's thallus and subsequent decomposition of thalli increases N available in the soil substrate and allows colonisation of nutrient poor substrates by other organisms (Vitousek, 1994). The biological crust on rocks exposed by retreating glaciers (formed primarily of crustose lichen) has been shown to greatly facilitate colonisation of these areas by tracheophytes by acting as pseudo-soil (Breen and Levesque, 2008).

Foliose and fructicose lichen compose of a very significant part of the diet of Arctic and sub-Arctic reindeer (*Rangifer tarandus*) which are an integral part of the way of life indigenous peoples such as the Sami in Scandavia or the Chukchi in Northern Asia (Roturier and Roue, 2009). Lichen forage is also important for other animals such as the Northern red-backed vole (*Myodes rutilus*) (Dokuchaev, 2009). Lichens are considered to be an important part of the ecology of polar invertebrates being both a food source and providing a sheltered habitat (Llano, 1948).

1.1.4 The economic importance of lichens

There is a long history of lichen exploitation by humans for medicinal, nutritional and economic value (see Llano (1948) for full review). Before the production of synthetic dyes whole shiploads of lichen were harvested for use in the European dying industry (Huneck, 1999). Lichen secondary metabolites have shown potential in the treatment of tumours and as anti-infection agents (Huneck, 1999). At the end of last century the cosmetic industry were harvesting over 2000 tonnes per year of *Pseudevernia furfuracea* and *Evernia prunastri* for use in perfumes (Huneck, 1999).

The ecological importance of the loss of lichen species

Finally it is worth noting that theories of species extinction predict that the loss of too many species would cause catastrophic, and irreparable with our lifetime, ecological damage. The Insurance Hypothesis predicts that to some extent a high biodiversity can buffer against the effects of an increased extinction rate. Biologically the differential response of species, which occupy the same

ecological niche, to a climatic stimulus has been shown to stabilise the loss/gain of community biomass (Leary and Petchey, 2009).

1.2. Ecological significance of temperature for lichen species

1.2.1 Evidence for the effect of temperature on the growth of lichens

Climate change in this last century has led to a general increase in annual global temperature (Serreze *et al.*, 2000). It has been hypothesised that the change in temperature has had a significant effect on the distribution of lichens on a continental scale. In Western & Central Europe, greater numbers of species, previously found in a more Southerly distribution (where they are found in warm temperate and subtropical ecosystems), have been documented at latitudes higher than they had been before (Aptroot and van Herk, 2007; van Herk *et al.*, 2002). For example, the epiphytic species *Flavoparmelia soredians*, which previously had a most Northernly limit in South England and was rare in the Netherlands before 1987 (completely absent after 1900), is now considered common in the Netherlands and its surrounding countries (Aptroot and van Herk, 2007).

Along with the spread of thermophilous species there has been a general decline in the number of psychrophilous lichen species in the same area (See Figure 1.2.1). Some studies have linked this change in lichen species distribution, directly to temperature changes towards the end of the 20th Century in Europe: for example van Herk *et al*. (2002) in the Netherlands and Lattman *et al.* (2009) in Sweden. Loss of terricolous *Cladonia* species in the Netherlands, although not directly linked to temperature, has been shown not to correspond to air pollution or land management (the other two major factors currently attributed to European lichen distribution change) and is most likely related to the boreo-alpine ecology of the species (Aptroot and van Herk, 2007; Hauck, 2009). The effect of temperature on the distribution of lichen species is thought to be more pronounced in higher latitudes meaning it is most likely an important factor in the ecology of Arctic lichens (Benedict, 1990; Hauck, 2009).

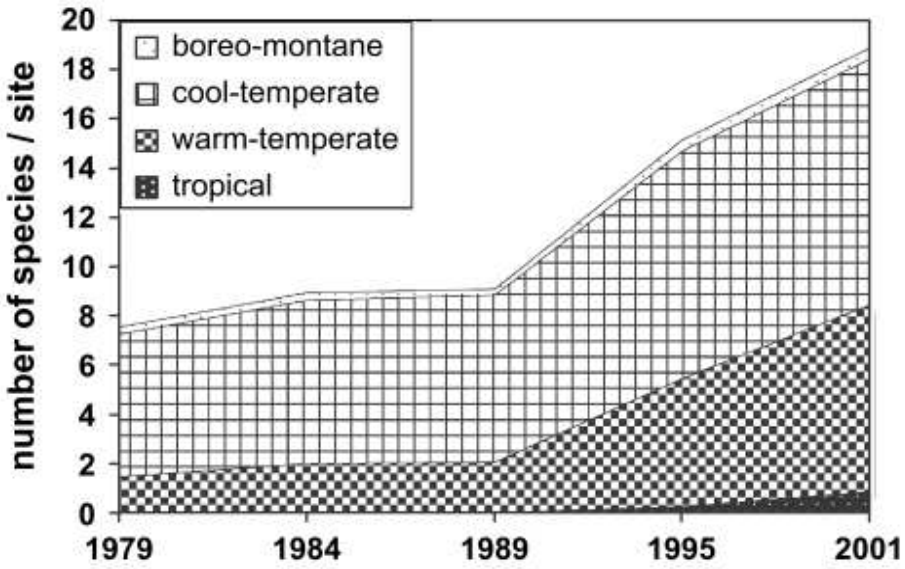

Figure 1.2.1: Average number of epiphytic lichen species found at Utrecht site, Holland. Species are separated according to the biome with which they are most closely associated. Other sites follow a similar trend exhibiting a rise in tropical and warm-temperate species over the last two decades. The key indicates the pattern used to split the total number of lichen species into groups, according the environment in which the lichen species is most commonly found. From Aproot and van Herk (2007).

1.2.2 How does temperature affect lichen growth?

It is well known that temperature affects photosynthetic rate according to reaction rate theory (Sharp, 1983). This also holds true for the metabolism of lichens. The temperature of a lichen thallus affects the rate of dark respiration (DR) and the rate of gross photosynthesis (GP); these two effects combined produce a response curve of net photosynthesis (NP). It is important to measure NP because it is directly correlated to growth-rate and biomass accumulation in lichen species, which is not only important physiologically, but can help predict the effect of the abiotic environment on the ecology of a lichen species (Benedict, 1990; Lepetz *et al.*, 2009; Palmqvist and Sundberg, 2000). If lichen biomass accumulation is retarded then reproduction can be affected, *Umbilicaria cylindrica* and *Lasallia pensylvanica* apothecum (a reproductive spore-productive structure) density increases with thallus size (Shimizu and Kubo, 2009) and in *Lobaria pulmonaria* reproductive effort is positively correlated with thallus age and size (Gauslaa, 2006). Hence factors that affect NP will also affect such factors as species dispersal capabilities and recruitment rates of lichen populations.

Ex situ studies provide valuable information on the physiological response of lichens to changing temperature, Figure 1.2.2 is a good illustration of the standard response of lichen

metabolism to changing thallus temperature. GP tends to exhibit a curved response, see Figure 1.2.2, to temperature, as expected with any photosynthetic organism (Lange, 2002; Sharp, 1982).

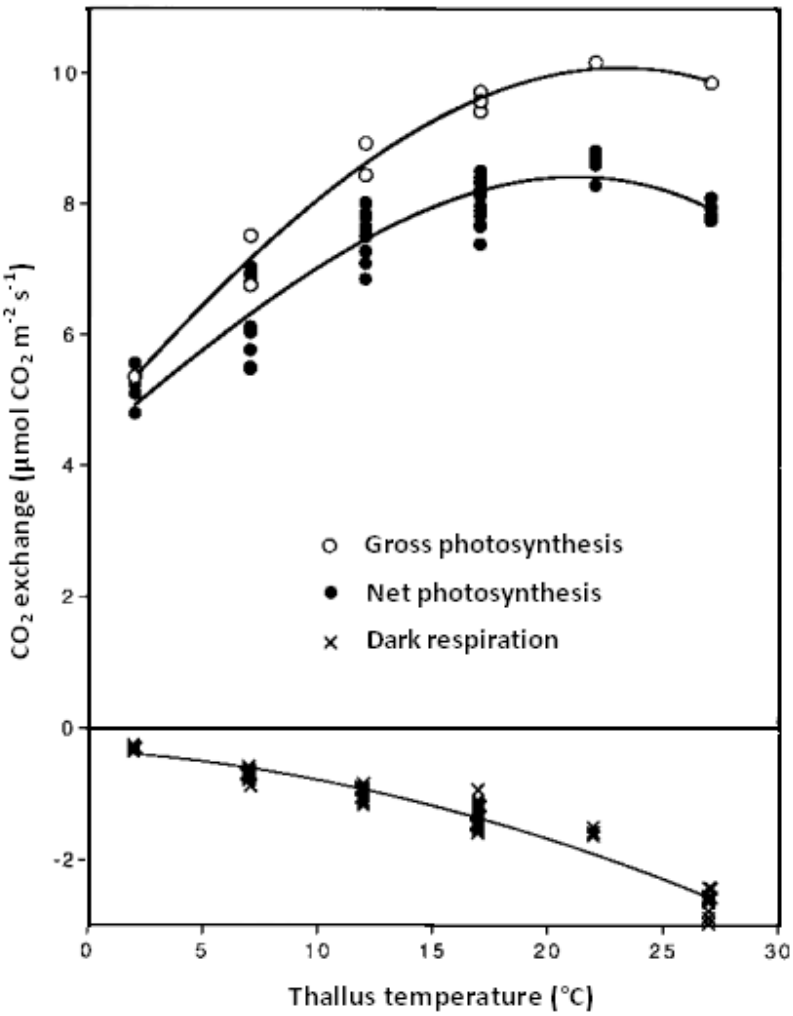

Figure 1.2.2: The effect of thallus temperature on the maximal rates of gross photosynthesis, net photosynthesis and dark respiration for the lichen species *Lecanora muralis*. From Lange (2002) – Figure 10.

The response curve is similar for most species, however the optimum temperature and the range within which photosynthesis occurs will differ according to species ecology, due to different temperatures of enzyme denaturation and lipid membrane destabilisation (Reiter *et al.*, 2008; Sharp, 1982). DR also responds to temperature, where thallus warming causes an acceleration in the rate of carbon dioxide (CO_2) evolution, most likely caused by the escalated rate of metabolic reactions with increased temperature, as predicted by Sharp (1982) (Harrisson *et al.*, 1986; Lange, 2002; Sundberg *et al.*, 1999). This is just a general response to temperature as the actual values of DR (in terms of actual

mols of CO_2 evolved) are species-specific and the shape of the temperature response curve will be related to the ecology of the species (Aubert *et al.*, 2007; Kappen, 2000; Lange, 2002). Figure 1.2.3 depicts the response of five different lichen species respiration rates to changing temperature, the differences between species are well illustrated as there are different respiration rates at the same temperature (Sundberg *et al.*, 1999).

The effect of temperature on both DR and GP produce a standard curved response of NP to temperature; where increasing temperature increases the rate of net CO_2 uptake (NP), to an optimum, then causes a rapid decline to net CO_2 release (see Figure 1.2.4). Figure 1.2.4 illustrates an example of where light and temperature produce a co-variant effect on the rate of CO_2 exchange, as the shape of the temperature response curve for *Xanthoria elegans* changes at different light intensities, this could have a significant effect on the productivity of a species *in situ* (Lange, 2002; Reiter *et al.*, 2008). As the response of GP and DR to temperature differs according to species so the optimal temperature of NP and the temperature range within which NP occurs are also species-specific (Reiter *et al.*, 2008).

Schroeter *et al.* (1996) highlight the importance of temperature in maintaining a positive carbon balance but in the study the importance of low temperature was paramount. At lower temperatures the low rate of DR means that less carbon-fixation has to occur in order to maintain net positive CO_2 exchange (lower light saturation point). The optimum photosynthetic photon flux density (PPFD) for NP is also lower at lower temperatures (Figure 1.2.5.). Hence increased air temperature during the winter would in fact decrease NP, due to increased DR limiting annual growth (Kappen *et al.*, 1995; Lange, 2002; Schroeter *et al.*, 1996).

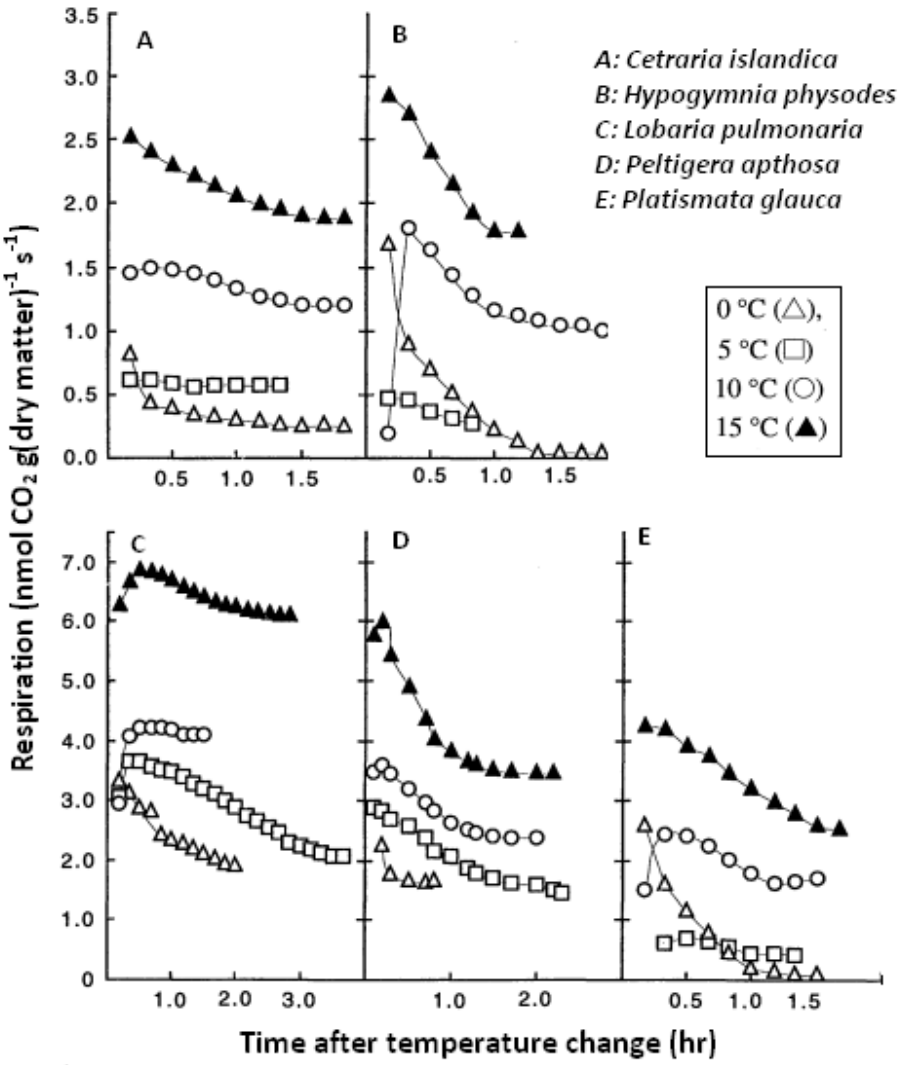

Figure 1.2.3: Changes in the rate of dark respiration over time after a change in temperature in five species of lichen. Temperature changes occurred in a stepwise manner starting at 0˚C and increasing in 5˚C increments. From Sundberg *et al.* (1999) – Figure2.

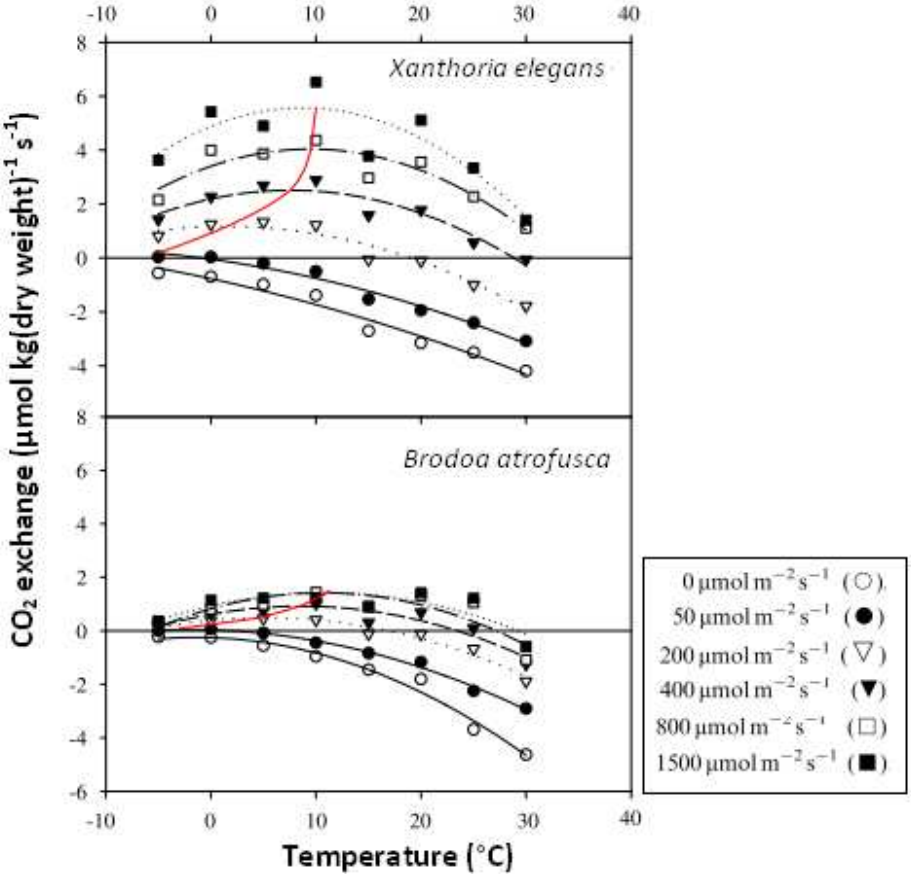

Figure 1.2.4: Rate of net CO_2 exchange against air temperature at different light intensities for two lichen species. From Reiter *et al.* (2008) – Figure 2.

1.2.3 The effect of temperature on lichen productivity and growth

The importance of the response of lichen thalli to temperature is highlighted in many *in situ* studies. These studies generally come to the conclusion that the temperature of an environment does not significantly affect lichen NP directly, i.e. through the reaction-kinetics mechanisms explained above, but through the interaction between temperature and other environmental factors that affect photosynthesis. A two year study of *Xanthoparmelia lineola* and *X. subdecipiens* growth rate by Benedict (1990) showed that greatest growth of lichen thalli was achieved during the warmest months of the year: May, June, July and August (as shown in Figure 1.2.6). Benedict (1990) also found a significant correlation between mean radial growths of lichen thalli and mean air temperature for each month but state that the correlation is due to the effect of temperature on thallus moisture as opposed to its direct effect on NP. Similar conclusions are made in Jonsson *et al.* (2008). The patterns evident in the *in situ* studies highlight the importance of long-term *in situ* experiments in studying the

ecological response of lichens to changing climate factors as the effect of temperature on the net productivity was not as simple as the interaction evident in *ex situ* studies.

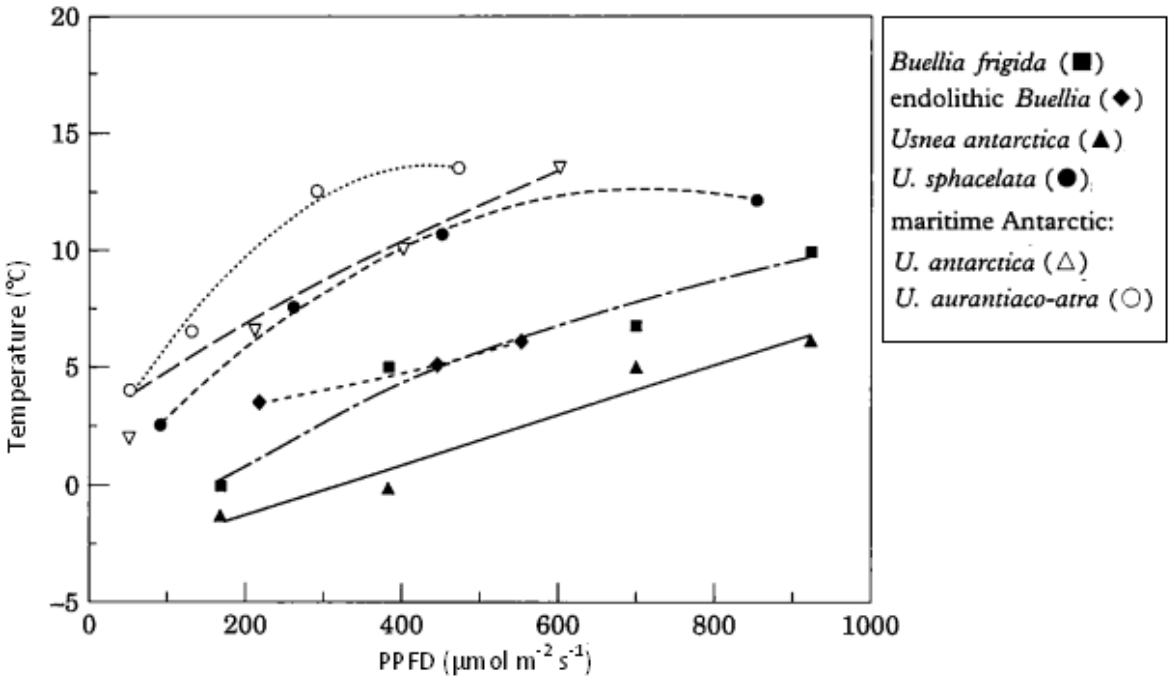

Figure 1.2.5: The relationship between optimum temperature for photosynthesis and PPFD in Antarctic lichens. Optimum temperature is the value which results in the greatest rate in CO_2 uptake at any given PPFD. From Kappen *et al.* (1995) – Figure 1.

1.2.4 Lichens and extreme temperatures

Specific lichen species have been shown to be very capable of surviving extreme temperatures and, in most cases, have an extremophilic nature (Kappen, 2000). Lichens tend to thrive mainly in areas where tracheophytes are not capable of establishing themselves or where extreme environmental factors prevent plants from out-competing them (Shaver and Chapin, 1991). The damaging effects of low temperatures have been recorded in some species where particularly cold winters can cause bleaching (photobiont death) (Showman, 1979). This is considerably less likely to happen in species specifically adapted to cold environments. However Showman (1979) suggests that lichen species susceptible to cold bleaching has more to do with morphology than ecology; i.e. that species which morphologically have higher surface areas are more likely do be damaged by extreme cold. The extent of damage from the process of freezing can be intensified in lichen species if the thallus is hydrated during the process (Larson, 1982; Larson, 1989).

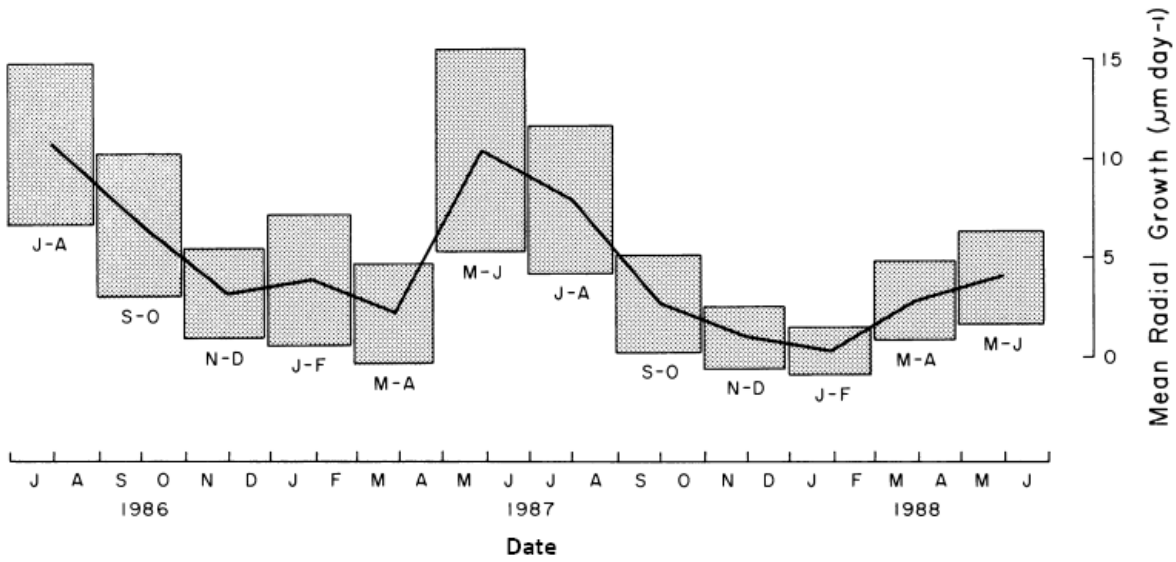

Figure 1.2.6: Mean bimonthly radial growth of *Xanthoparmelia* spp. thalli over a two-year period. The line denotes the actual mean growth, the horizontal width of the shaded block denotes duration of measurement period and the vertical length is the standard deviation within said period. From Benedict (1990) – Figure 2.

The diurnal and seasonal cycling of weather also has an effect on lichen survivability. Although most lichens are capable of surviving being frozen, extended periods where lichen are subjected to air temperatures that cause them to freeze and then thaw can cause substantial leaching of carbohydrates (Kappen *et al.*, 1995; Tearle, 1987). This can occur during diurnal cycles in the winter in high latitudes or altitudes where lichen will freeze at night and thaw during the day (Davey *et al.*, 1992). Potentially, in this case, constant freeze-thaw cycles would be considerably more damaging than extended cellular freezing. Also, during thawing events respiration rates of lichen thalli rise dramatically and can result in low rates of NP even during optimal conditions; repeated freeze-thaw cycles can cause a dramatic decrease in carbon gain through this effect (Davey *et al.*, 1992; Kappen *et al.*, 1995). It is worth noting that some experiments have found that snow and ice cover can act as a buffer to diurnal freeze-thaw cycles and protects lichens from damage associated with extremely low temperatures and extended periods of freezing and thawing (Bjerke, 2009; Kappen *et al.*, 1995; Korner, 1999).

1.3 Thallus hydration and growth

In a similar fashion to temperature the moisture content of a lichen thallus has a profound effect on the annual NP and growth of an individual lichen. Benedict (1990) suggests that 92-93% of annual growth variation, of *Xanthoparmelia lineola* and *X. subdecipiens* in the Colorado Front Range, is due to variation in snow-free thallus moisture and day-time duration. In an *in situ* study of *Lecanora muralis* thallus moisture content was shown to be the most limiting factor of annual NP, as even in times of saturating light and suitable temperature maximal potential NP was rarely reached (Lange, 2002); this circumstance is thought to be due to the effect of non-optimal thallus moisture content on NP (Figure 1.3.1). Many other studies have concluded that in alpine and arctic environments thallus moisture content is a greater factor in determining annual NP *in situ* than temperature (Reiter *et al.*, 2008; Sonesson, 1989; Uchida *et al.*, 2006).

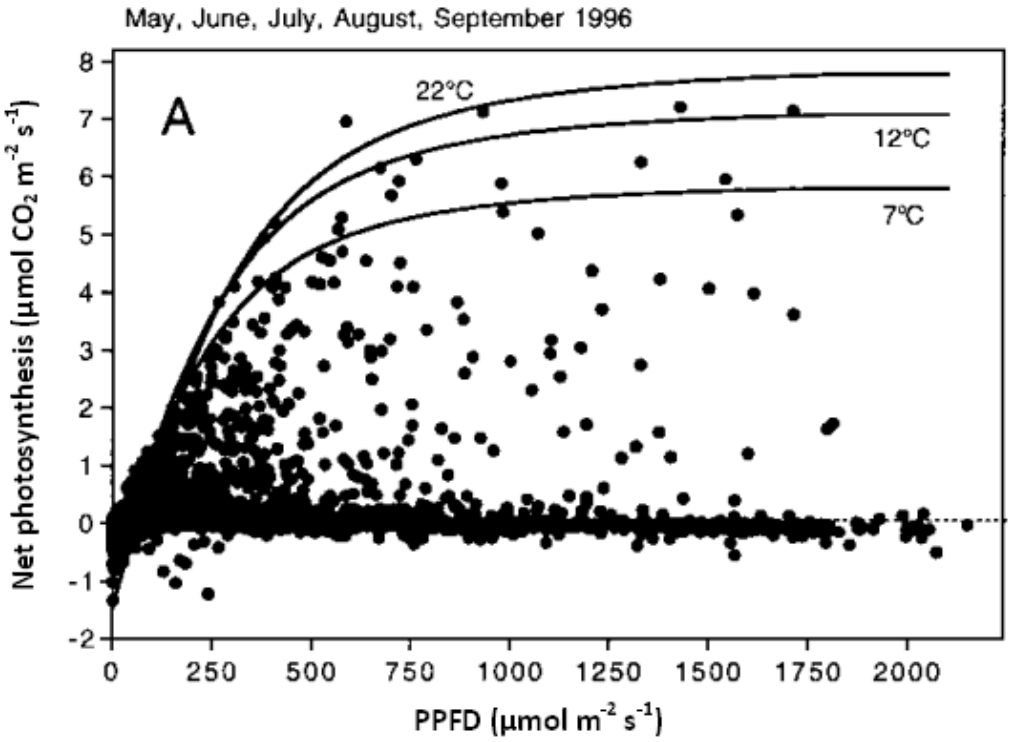

Figure 1.3.1: Figure displays series of *in situ* recordings of net photosynthetic rate of *Lecanora muralis* and the PPFD at the time of measurement. The lines indicate optimum response curves at 7˚, 12˚ & 22˚C created under laboratory conditions. Note that in almost all cases NP is sub-optimal at any given PPFD and in many cases is negative. From Lange (2002) – Figure 17.

1.3.1 The interaction between thallus hydration and metabolic activity

Both DR and GP rates vary according to the hydration state of the thallus. DR response to thallus moisture content follows saturation type kinetics; i.e. as metabolic activity reactivates after desiccation respiration increases until after a certain thallus water content, thereafter the rate of DR levels off (Figure 1.3.2 & 1.3.3). The impact of rehydration of a thallus on its DR rate differs according to how the thallus was hydrated: immersion in water (sudden rehydration) has been shown to lead to exaggerated DR rates for up to 10 hours after immersion, the phenomenon is known as "resaturation-respiration" (Sundberg *et al*., 1999). The amplification of the rate of DR caused by rehydration has been demonstrated to increase when the thallus is exposed to longer periods of desiccation (Brown et al., 1983). Rehydration by humid air does not present the same problem for lichen and results in a steady response of DR (Lange and Green, 1996; Nash, 1996). The reason for the different rates of DR between the two treatments is stated to be unknown, but it is known that the increased respiration rate is fuelled, in *Xanthoria elegans*, by gluconate-6-phosphate stored within the thallus during the desiccation process (Aubert *et al.*, 2007).

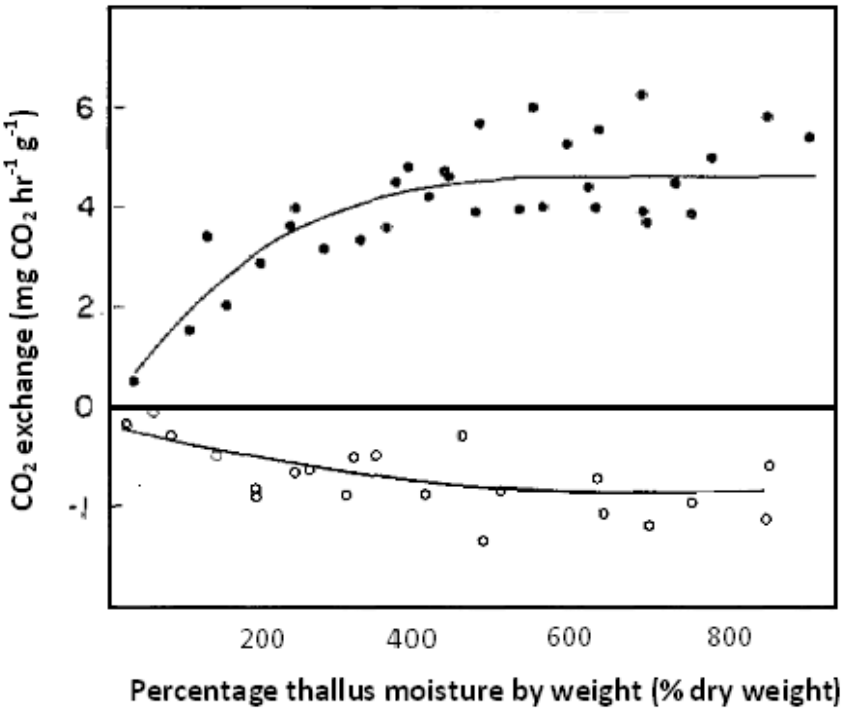

Figure 1.3.2: Response of net photosynthesis and dark respiration of *Nostoc commune* and change in degree of thallus hydration. Positive values are indicative of photosynthesis, negative of dark respiration. From Coxson *et al.* (1983) – Figure 6a.

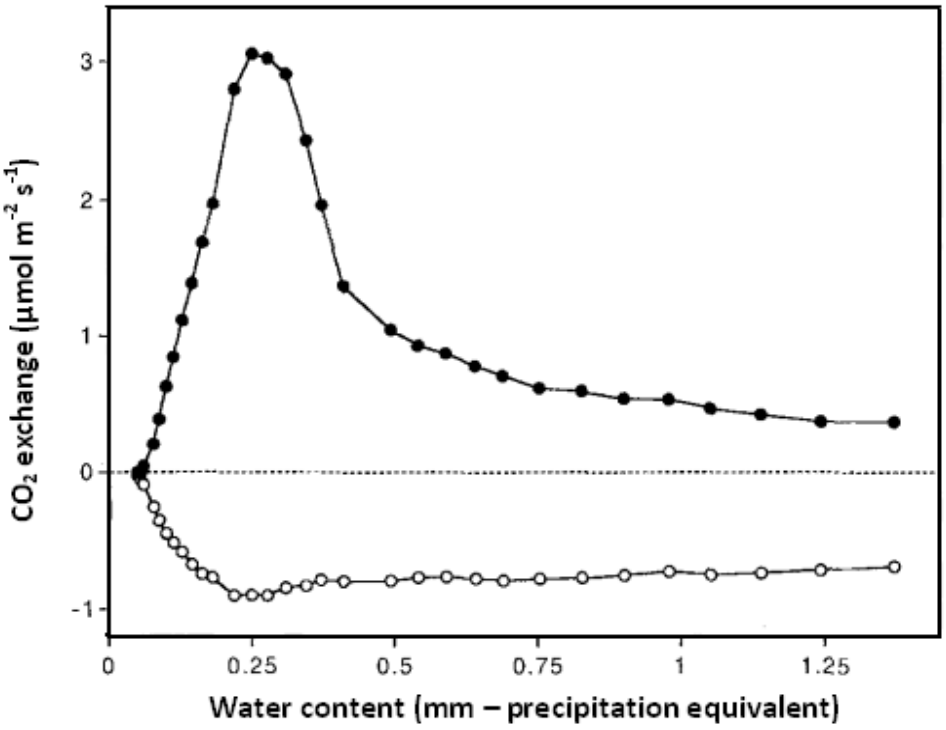

Figure 1.3.3: The dependence of net photosynthesis (positive values) and dark respiration (open circles – negative values) on thallus water content for the lichen species *Xanthoria calcicola*. Data were recorded at 17˚C and 1000 µmol m^{-2} s^{-1} PPFD. From Lange and Green (1996) – Figure 1.

As shown in Figure 1.3.3, NP follows a curved pattern in response to changing thallus-water content (Lange, 2002; Lange *et al.*, 2001; Nash, 1996). The current theory is that the curve is the result of a two-part response (Harrisson *et al.*, 1986; Lange *et al.*, 2001):

Firstly, the increase at the beginning of the curve is the result of the easing of desiccation stress (sub-saturation depression). As desiccation of the lichen thallus causes the photobiont to cease photosystem II activity, to protect itself from photodamage (see section 1.4), the initial increase in NP is thought to be due to the reversal of this process along with the general reactivation of metabolic pathways (Lange *et al.*, 2001; Lange *et al.*, 1988).

Secondly, the increase in NP is followed by a drop to and eventually a levelling at a steady rate of CO_2 exchange; whether this is positive or negative. The decline of NP at above optimal thallus moisture content is thought to be due to a phenomenon known as 'supra-saturation depression'. Lichens are not plants and lichen thalli do not have stomata and in order for CO_2 to reach the photobiont, gas must travel through the mass of mycobiont hyphae in the lichen thallus. Normally

there are air pockets between the hyphae by which gases can reach the photobionts. When the thallus becomes more hydrated the hyphae swell, which closes these air pockets, and the channels fill up with water. As the diffusion resistance of CO_2 influx of water is considerably greater than air, the movement of CO_2 through the lichen thallus towards the photobiont is retarded by above optimal water contents (Cowan *et al.*, 1992; Harrisson *et al.*, 1986). As the electron transport rate and not just the CO_2 exchange rate was also found to be depressed by high water content, for both green-algal and cyanobacterial photobionts, it has been suggested that the depression of CO_2 exchange is directly correlated to an actual depression of NP; as opposed to a recycling CO_2 made available by thallus respiration (Lange *et al.*, 1996). The diffusion resistance increases with higher temperature and at higher temperatures lichen thalli exhibit greater "super-saturation depression" (Cowan *et al*., 1992). It has been shown that the effects of "super-saturation depression" can be ameliorated by increasing the CO_2 concentration in the air (Lange, 2002).

The effect of sub and supra-saturation depression of lichen NP is very significant. Originally 'super-saturation depression' was considered to be an artefact of unreal water conditions in laboratory experiments. However many experiments have shown that supra-saturation significantly reduces NP in field experiments; including those undertaken in arctic environments (Kappen and Breuer, 1991; Lange and Green, 1996; Lange *et al.*, 2001; Reiter *et al.*, 2008; Schipperges, 1992). Laboratory experiments have shown that super-saturation can depress NP by up to 80% (of maximal NP at same light and temperature conditions) under saturating light conditions though figures tend to be around 50% for most species at lowest light levels (Kappen and Breuer, 1991; Lange and Green, 1996). Lange (2002) has shown that with *L. muralis*, 90% of maximal NP occurs between water contents of 0.44 and 0.64 mm (mm is the amount of water taken up by a thallus as precipitation – 1mm is equal to one litre of water per metre^2) where the range of photoysnthetically active thallus moisture content is approximately a third of the total range of photosynthetically active moisture content (Figure 1.3.4). Some species are known to not be affected by supra-saturation depression; in these species NP exhibits a saturation type response to thallus hydration status as illustrated in Figure 1.3.2 (Coxson *et al.*, 1983; Lange *et al.*, 1997).

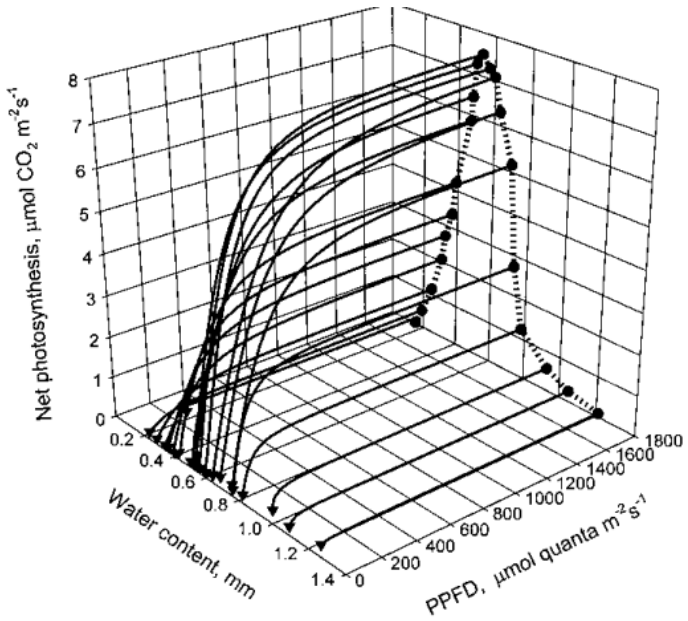

Figure 1.3.4: Light response curves of net photosynthesis, for *Lecanora muralis*, at different thallus water contents. Water content is given in mm precipitation equivalent. From Lange (2002) – Figure 2.

The amount of time a lichen thallus spends in a photosynthetically active state (wet active time) within a period of time is very closely related to the net productivity and the growth of the thallus within that period (Benedict, 1990; Lange, 2002; Palmqvist and Sundberg, 2000; Reiter *et al.*, 2008). Also the production of the important protective secondary metabolites, requires lichens to be hydrated and in order to survive adverse light, heat and hydration conditions lichen must be metabolically active before stress event in order to produce protective compounds (Bjerke *et al.*, 2005a; Solhaug *et al.*, 2003).

1.3.2 Climatic factors that affect thallus hydration

It is important to remember that lichens are poikilohydric and as such the thallus moisture content of an individual is dependant mostly upon the climatic conditions (Nash, 1996). However the effect of climatological factors on thallus hydration state differs between species (Gauslaa and Solhaug, 1998). The pattern, of how hydration state is affected by climate factors, is strongly influenced by the morphological type of the species (Hartard *et al.*, 2009). Similarly the rate at which species lose or gain water, at different relative-humidities and temperatures, is closely related to the functionality of the species; i.e. hydrophilic species show a reduced ability to retain thallus moisture,

at a low relative humidity, when compared to xerophilic species (Liden *et al.*, 2010). Further more the morphology of a species may influence hydration status by affecting surface area exposure to said climatic conditions; such as the tendency to form cushions or mats or more importantly with lichen epiphytic or terricolous etc. (Sonesson, 1989; Zotz *et al.*, 2000).

The link between climatic factors and lichen thallus moisture content *in situ* is extremely complex. Few studies find direct correlations between amount of precipitation and lichen growth as precipitation tends not to be a good indicator of thallus hydration status (Benedict, 1990). Uchida *et al.*, (2006) argues that under snow free conditions thallus hydration status is well predicted by precipitation, but within the results the great variation of snow-free precipitation is not reflected by net production (Figure 1.3.5).

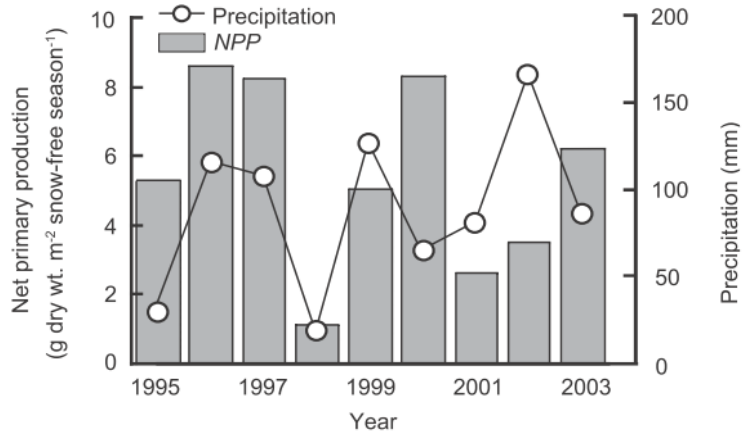

Figure 1.3.5: Net primary production of lichen species *Cetrariella delisei* and the amount of precipitation during the snow-free season. Unfortunately a correlation analysis was not performed. From Uchida *et al.* (2006) – Figure 6.

The lack of direct correlation between thallus hydration status and precipitation may be due to the multitude of ways that lichens may become hydrated, other than through rain-water. Lichen species have been shown to be able to reach metabolic active water contents through air moisture (for a review and evidence as to the ecological importance of the phenomenon see Lange *et al.* (2001)). In fact lichen thalli are rarely found in a photosynthetically active hydration state *in situ* at a relative-humidity (RH) lower than 90% (Schroeter *et al.*, 1996). High air moisture has been shown to considerably increase the length of time which lichen thalli are in a metabolically active hydration

state in arctic and alpine studies and as such may play an important part in NP and consequently annual net production and annual growth (Sonesson *et al.*, 1989; Uchida *et al.*, 2006). Rehydration to an equilibrium state through air moisture takes a long time, 30 hours in *Cladina mitis* and *C. rangiferina* at 90% RH, and most of the time is not achieved before atmospheric RH changes (Heatwole, 1966). Different species exhibit diverse capabilities to utilise air moisture as a water source for metabolic activity, on one end of the scale purely cyanobacterial lichen species have no documented cases of rehydration from atmospheric moisture while *U. longissima* rehydration through this mechanism forms a significant proportion of total wet-time(Cabrajic *et al.*, 2010). Lichens have also been shown to rehydrate and become metabolically active through: snow cover (Kappen and Breuer, 1991; Kappen *et al.*, 1998; Kappen *et al.*, 1995; Schroeter *et al.*, 1996), dew fall (Lange *et al.*, 2001; Lange *et al.*, 1986) and snow melt (Kappen *et al.*, 1998). Furthermore total precipitation does not give an indication of how the rain fell; long periods of light rainfall are thought to keep lichen metabolically active longer than short deluges within which the same, or more, precipitation falls (Benedict, 1990).

As thallus water content is thought to be one the most important factors in net annual production of lichen and it is considered particularly difficult to measure *in situ*. A recent effort has been made to predict thallus moisture content of a species using climatic factors using information based on older *in situ* experiments which have shown that thallus water content is significantly and strongly correlated to short term (> 24 hour) changes in relative humidity (Heatwole, 1966). More recent studies consider the water potential of the air to be the most important factor in estimating thallus moisture content (Cabrajic *et al.*, 2010; Jonsson *et al.*, 2008). Water potential of the air is a result of the interaction between temperature and relative humidity and potentially provides a mechanism that ratifies the hypothesis made by Benedict (1990), where temperature significantly affects growth due to interaction with hydration induced photosynthetically active period. Experimental studies have found that in epiphytic lichen active wet time can be successfully modelled through calculating air water potential (Jonsson *et al.*, 2008) and that in terricolous mat forming lichen the most important factors in determining on thallus hydration state is evaporative forcing of the local atmosphere (Gaio-Oliveira *et al.*, 2006). The relationship between active wet-time and NP is complicated by the slower activation of photosystems, in some species, by atmospheric moisture as opposed to the fast activation by liquid water; in one study even though water vapour and liquid water contributed equally to thallus active wet-time, liquid water contributed significantly more to realised NP (Liden *et al.*, 2010). It is important noting that in both the older *in situ* studies and the modelling scenarios NP is strongly affected by daily changes in RH independently of the season of

measurement; this could suggest that lichens are capable of significant production outside of the peak vascular growth season.

1.4 Effect of light related external environment factors on lichen productivity

1.4.1 Photosynthesis and light

Light regime is important in determining NP. In higher latitudes lichen growth occurs mostly in the summer period (Benedict, 1990; Kappen *et al.*, 1998; Schroeter *et al.*, 1995; Uchida *et al.*, 2006). Benedict (1990) suggests that the greater growth in the summer is due to the longer day length providing more time for growth to occur; this maybe more true for the highest latitudes, when compared to lower latitudes, as day length is more variable closer to the poles (Figure 1.4.1). The magnitude of photosynthetically active radiation (PAR) available for photosynthesis also has an effect and light intensity has been shown to be a limiting factor in achieving higher NP in lichens in the winter (Schroeter *et al.*, 1996). Light intensity also plays an important role in determining the hydration status of polar lichens: firstly day length and light intensity affect the temperature of an ecosystem by providing solar-radiation which heats up an environment increasing the rate of water loss of of thallus and reducing atmospheric water availability (which has already been shown to affect wet-active time, and secondly light intensity has been shown to be an important part of snow melt which is an important source of thallus moisture for polar lichen (Kappen *et al.*, 1998; Wild, 2009). Light availability is considered to be the most important factor in determining NP when lichens are at optimum metabolically active thallus moisture contents (Dahlman and Palmqvist, 2003; Palmqvist and Sundberg, 2000).

As one would expect, many laboratory experiments have associated higher photosynthetic photon flux density (PPFD) with increased NP in lichens. Most studies show saturation type kinetics (Figure 1.3.4), assuming all other factors stay the same, where higher light intensity increases NP until light is saturating and no longer the limiting factor (Green *et al.*, 1998; Lange, 2002). From the fluorescence measurements made in Green *et al.* (1998) it can be seen that GP and NP mirror each other with changing PPFD, from this it can be assumed that PPFD does not affect rate of DR within non-damaging intensities. The light saturation value changes according to ambient temperature and thallus moisture content (Lange, 2002). Earlier the change in the light compensation according to changing temperature was highlighted. As temperature affects the rate of DR, the rate of carbon fixation required to produce a positive CO_2 balance increases with increasing temperature (Lange, 2002; Sonesson *et al.*, 1989). However temperature also affects the efficiency of photosynthesis, measured as the maximum apparent quantum use efficiency (Lange, 2002). The variation of both DR and efficiency with temperature leads to a change in the light level required to reach optimal NP at any given thallus moisture. Thallus moisture content therefore affects the light saturation point of NP in lichen (Figure 1.4.2).

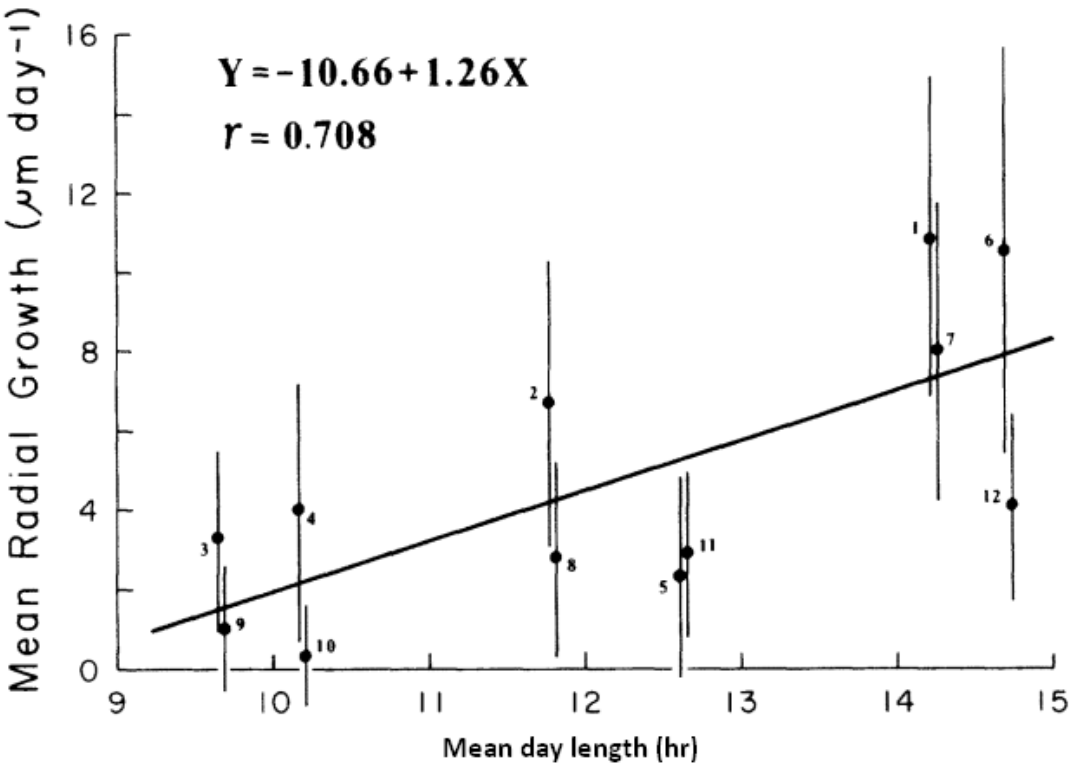

Figure 1.4.1: Mean radial bimonthly growth of *Xanthoparmelia lineola* & *X. subdecipiens* thalli compared to the day length (in hours of day-light) during the period of measurement. Correlation analysis finds the data to be significantly positively correlated (*P*= 0.01). From Benedict (1990) – Figure 5.

The relationship is complex and is related to the maximal ability of the thallus to photosynthesise at certain moisture contents; i.e. saturation point is low at low moisture contents as not all photosystems are active, at optimal moisture content saturation point is at its highest as photosynthetic capacity is highest and at higher moisture contents supra-saturation depression decreases photosynthetic capacity as there is less CO_2 available so less light is required to reach saturation point (Lange, 2002). Optimal and saturating light conditions are known to be different for different species and the difference can be related to species ecology; i.e. species from more exposed habitats will be expected to have higher saturating light intensities (Nash, 1996). For example in a comparison of two epiphytic lichen from the same habitat (Northern Swedish birch forest) the species that occupied a higher layer of the canopy (*Parmelia olivacea*) has a higher light saturation point (500 μmol m^{-2} s^{-1}) than the species that inhabited a lower layer (*P. ambigua* – 300 μmol m^{-2} s^{-1}) (Sonesson *et al.*, 1989).

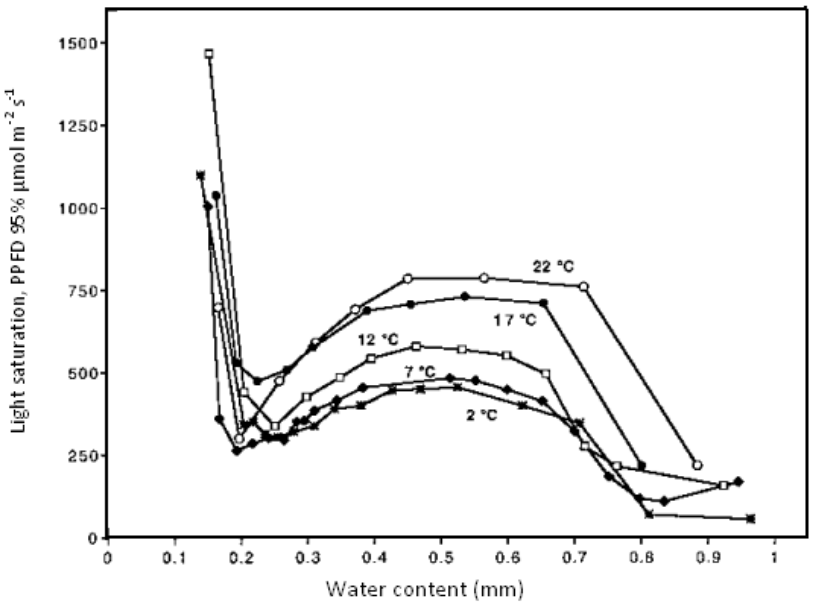

Figure 1.4.2: The light intensity at which 95% of theoretical maximal net photosynthesis (i.e. the light saturation point) is reached at a range of thallus water contents and air temperatures; for the species *L. muralis*. From Lange (2002) – Figure 6.

1.4.2 Light stress

As occurs with higher plants, doses of light which are of considerably higher intensity than a thallus is used to have been shown to lead to photoinhibition in lichens (a light induced reduction of photosynthetic capacity) (Bartak *et al.*, 2004; Demmig-Adams *et al.*, 1990). The decrease in capacity caused by high-light doses has been hypothesised to be due to contribution from two factors: firstly capacity is reduced by actual damage to the photochemical apparatus and secondly due to the dissipation of excess excitation within lichen light-harvesting complexes (Demmig-Adams *et al*., 1990). Again hydration status and air temperature affect the intensity of photoinhibition. Temperature has been shown to have an additive effect with high light conditions and when the two are combined produced larger reductions of photosynthetic capacity than either does individually (Gauslaa and Solhaug, 1999). Lichen thalli have also been shown to be capable of faster recovery of lost photosynthetic capacity if they are desiccated prior to exposure to photoinhibiting light levels; this is most likely due to decoupling of the electron transport chains in the photobiont light-harvesting systems allowing greater levels of energy dissipation (Demmig-Adams *et al.*, 1990). Lichenised algae, within the thallus of *Ramalina yasudae* , exhibited no photosystem II (PSII) activity when they were in

a desiccated state (Kosugi *et al.*, 2009). The cessation of PSII activity is thought to be an important mechanism to protect the photosynthetic apparatus from damage caused by reactive oxygen species (Kosugi *et al.*, 2009); the process is hypothesised to occur through a reversible mechanism of induced changes in the morphology of chlorophyll pigments (Heber, 2008; Heber *et al.*, 2010). Desiccation also increases the amount of light scattered by the fungal cortex and provides a significant screen against over-stimulation of the algal photosystems during desiccation stress (Kosugi *et al.*, 2009). Long-term irradiation of thalli with photoinhibitory light intensities has been shown to decrease photosynthetic capacity more than single bursts of the same intensity; inclusion of further bursts of irradiance following a prolonged burst do not significantly affect the reduction of capacity (Bartak *et al.*, 2008).

Light stress is avoided in lichen photobionts either through photochemical or non-photochemical quenching (Bartak *et al.*, 2004; Bartak *et al.*, 2008; Demmig-Adams *et al.*, 1990). Non-photochemical quenching is achieved in green-algal photobionts, as in higher plants, through the zanthophyll cycle (Bartak *et al.*, 2004; Heber, 2008). The ability to resist photosynthetic capacity reduction in lichens has been shown to be directly related to the size of the xanthophyll pool within the thallus (Bartak *et al.*, 2004; MacKenzie *et al.*, 2002).

Increases in UV-B radiation have been shown to have insignificant effects on the growth rate or the NP of lichen *ex situ* (Bjerke *et al.*, 2005b; Larsson *et al.*, 2009). Lichens which have been extracted of their UV protective pigments have been found to rapidly re-produce them when exposed to UV-B radiation (Solhaug *et al.*, 2003). This suggests that predicted rises in UV-B radiation levels in the Arctic over the next few decades are unlikely to significantly affect indigenous lichen ecology (http://www.eoearth.org/article/Future_changes_in_ultraviolet_radiation_in_the_Arctic).

1.5 Within species variation of the net photosynthetic rate of lichens due to environmental factors

1.5.1 Measuring the significance of seasonal fluctuation of NP to the contribution of lichens, to net ecosystem productivity

The importance of the arctic ecosystem as a global carbon sink was highlighted in the first section of this chapter. The potential for lichens, which make up a substantial part of the biomass of sub-arctic boreal region ecosystems, to contribute significantly to net ecosystem production, has not been suitably described. The potentially inaccurate representation of the significance of lichen contribution of lichens to arctic-heath net ecosystem productivity is due to the seasonal variation of the NP of lichens (Campioli *et al.*, 2009).

It has been shown that the NP of lichens is significantly, and directly, affected by the external environment of a thallus. The factors, which significantly affect the NP of lichens, vary over the course of a year and many species of lichens have exhibited a seasonal variation in thallus NP (Lange, 2003a; Lange, 2003b). The seasonal change in the NP of lichens can be attributed to variation of the external climatic factors that have been found to significantly affect NP in laboratory experiments: i.e. the temperature, water potential of the atmosphere and light intensity of the surrounding environment (Cabrajic *et al.*, 2010; Jonsson *et al.*, 2008). Due to the seasonal variation of the NP of lichens, it is important to measure net productivity of a species during the correct time of year, in order to ascertain the maximum contribution of the species to net ecosystem productivity. The time of peak photosynthetic rate in arctic lichens had previously been considered to be the same as for vascular plants; which reach maximal net productivity in the mid-summer weeks, and it was subsequently assumed that non-vascular productivity did not significantly contribute to net ecosystem productivity in arctic heath ecosystems (Campioli *et al.*, 2009; Lange *et al.*, 1998; Williams *et al.*, 2000). It had been assumed, as higher air temperatures result in greater rates of NP in laboratory studies, that the greatest productivity in lichens is achieved during the warmest period of the year. However more recent studies are suggesting that, *in situ*, hydration status of a lichen thallus plays a more significant role in determining NP of a lichen thallus than temperature and that there are periods of time in which lichens are capable of reaching maximal net photosynthetic rate throughout the snow-free season (Lange, 2002; Lange, 2003b). In the arctic-heath species *N. arcticum*, the contribution of temperature, while thalli were not optimally hydrated, in determining the rate of NP of a thallus was less than 1 %, ANOVA results found this contribution to be non-significant, between June to October (Sonesson *et al.*, 1992).

The results, of studies of the factors that affect the NP of lichens over the course of the year, would suggest, as temperature is not as significant a factor in determining the NP of lichens *in situ* as originally thought, that the period of greatest productivity of arctic lichens is in fact during the months during which thalli can achieve the greatest wet active time; it is important to note that this does not necessarily correspond to the period of the year with the greatest precipitation, as it has been outlined earlier in this report that precipitation does not accurately predict active wet time. Furthermore the low air temperature in arctic-heath ecosystems, which suppresses DR and results in significant rates of NP in cold-adapted lichens, during the early spring and late autumn, could potentially allow lichens within the ecosystem to significantly contribute to NEP outside of the viable growth season of vascular plants when it is too cool for vascular plants to achieve NP; as occurs in the alpine environments in the Antarctic (Reiter *et al*., 2008).

The seasonal change in productivity of reindeer lichens (*Cladina stellaris*, *C. mitis*, *C. rangiferina* & *C. arbuscula*), which comprise a substantial part of arctic-tundra and heath ecosystem biomass and almost all of the lichen biomass in late successional stage arctic tundra ecosystems, has not been studied in populations from the Fennoscandian region (Arseneault *et al.*, 1997; Gaio-Oliveira *et al.*, 2006). If any species of lichens significantly contribute to arctic NEP then it is most likely the reindeer lichens and by determining which factors significantly affect the NP of *Cladina* species *ex situ*, it would be possible to predict what time of the year they achieve their maximum NP. If the maximum NP of *Cladina* species is achieved outside of the vascular peak growth season lichens could truly have been providing a significant, over-looked proportion of arctic NEP.

1.5.2 Acclimation

Lichens exhibit a high level of adaptation to variation of climatic factors within the environment in which they are found. Lichen species exhibit two distinct types of within species variation of photosynthetic characteristics, which result from adaptation to the specific climatic conditions of environment each individual inhabits:

Firstly lichen species exhibit phenotypic differences, such as different morphological characteristics, between populations which exist in different ecotypes; e.g. high altitude and low altitude populations. Difference in the capacity of thalli to withstand photoinhibitory stress is evident in sun- or shade- adapted species have been found in lichen populations, lichen thalli from shade-adapted individuals tend to show traits such as higher chlorophyll *a* (Chl *a*) contents and lower photo-protective compounds, such as UV-B-protecting microsporine-like amino acid derivatives, than sun-

adapted individuals of the same species; see Table 1.5.1 (Kappen, 2000; Kershaw, 1985; MacKenzie *et al.*, 2002; Vrablikova *et al.*, 2006).

Table 1.5.1: Concentrations of pigments and UV-B absorbing amino-acid derivatives in sun ecotype and shade ecotype thalli, of the lichen species *Umbilicaria aprina* from continental Antarctica. Values given in mg g (dry weight)$^{-1}$. From Kappen (2000) Table L.

	Sun form	Shade form
Chlorophyll *a*	1.8 ± 0.6	8.5 ± 2.3
Chlorophyll *b*	4.2 ± 0.1	2.2 ± 0.4
Phenophytin a	3.2 ± 0.2	1.2 ± 0.6
Neoxanthin	2.4 ± 0.6	1.3 ± 0.1
Violoxanthin	0.5 ± 0.3	0.5 ± 0.2
Zeaxanthin	1.9 ± 0.2	1.2 ± 0.2
Lutein	8.4 ± 1.2	5.9 ± 0.4
Mycosporine-glycine	0.8 ± 0.03	0.58 ± 0.15

The process by which the chlorophyll content of a thallus, of the species *Parmelia pastillifera*, is reduced in 'sun-adapted' individuals, is by a photo-destructive mechanism, where exposure to high light intensities directly causes the breakdown of chlorophyll; as opposed to genetic variation between populations (Tretiach and Brown, 1995). It has been shown that in *C. rangiferina* the optimum temperature of photosynthesis is higher in thalli from a more Southern population when compared to the optimum temperature of thalli from a northern population (Adams, 1971). It has not been shown, to my knowledge, whether similar differences in the photosynthetic capacity of lichen thalli, from different populations, occurs in populations which are more geographically separate from one another, for example do thalli of *C. rangiferina* from an arctic-tundra ecosystem in North America have the same photosynthetic response to temperature as species from the same environment in Europe. Differences in the photosynthetic characteristics of lichens from different populations has been shown, by isoenzymatic analysis, to not be due to genetic variation between thalli, and is suggested to be driven by climatic factors during thallus development (Kershaw *et al.*, 1983; Macfarlane *et al.*, 1983; Tretiach and Brown, 1995).

Secondly seasonal cycles of temperature and light intensity result in adaptation of thalli to their immediate surrounding environment; which results in terms such as winter - or summer adapted - thalli. Accumulation of important photo-protective compounds within lichen thalli has been shown to be cyclical within the year: *L. pulmonaria* thalli from habitats where they are subjected to

different light intensities through the year such, such as temperate forest where light may break through the canopy, had greater pool of xanthophyll cycle pigments in spring than autumn measurements, whilst pigment pool's of members of the same species from closed canopy forests did not change significantly (Mackenzie *et al.*, 2002). In *Xanthoria parietina* the photoprotective protein parietin is produced by the mycobiont. Parietin screens photosynthetically active radiation (PAR) and is an important protectant against photoinhibition, the production of parietin by the mycobiont is a response to exposure to PAR and there is evidence that production is a seasonal response to changing light intensity (Vrablikova *et al.*, 2006). Season variation of climatic conditions also results in photosynthetic plasticity; which is defined as fluctuation in photosynthetic characteristics such as maximum capacity, apparent efficiency and optimum temperature of photosynthesis (Brown and Kershaw, 1986).

The maximum mean rate of NP of lichens of the genus *Peltigera*, from a Canadian forest habitat, has been shown to be lower in thalli collected during full winter conditions than thalli collected during spring of summer from the same population (Figure 1.5.1), furthermore optimum temperature of photosynthesis in winter adapted thalli were ~5˚C while mid-summer adapted thalli reached maximal NP at 30˚C (Kershaw, 1977). It has been shown that in *Peltigera rufescens* that the kinetic parameters of an important enzyme in the photosynthetic process (fructose- 1, 6-bisphosphate), reversibly, change when exposed to variable temperatures, in such as way that it could account for the fluctuation of the optimum temperature of photosynthesis through the year (Brown and Kershaw, 1986). In both *C. stellaris* and *C. rangiferina* a similar drop in photosynthetic capacity, to that found in lichens of the *Peltigera* genus, has been found between winter and non-winter adapted individuals, it was concluded that the reduction in maximal rate of NP, in these species during the winter, was due to a reversible uncoupling of some photosynthetic units in the thalli that was a response to reduced photoperiod and low temperatures (Macfarlane *et al.*, 1983).

The acclimation response of lichens is a very important consideration when performing comparative physiology experiments. Unless one is actually considering differences between thalli, of the same species, adapted to specific conditions then a pre-experimental conditioning is required during the storage process. Exposure of all specimens to the same set of conditions prior to analysis is crucial in order to remove bias. It is also important to distinguish between thalli that exist in different ecotypes when considering the contribution of a species to ecosystem productivity, as they will often have different temperature optima and maximal photosynthetic capacity, although this factor can often be removed by providing rate of NP on a weight of chlorophyll basis (Macfarlane et al., 1983; Tretiach and Brown, 1995). Finally the ability of a species to photosynthetically adapt to a changing

environment, by exhibiting photosynthetic plasticity, has been suggested to reduce the negative impact of predicted climatic warming on the distribution of species (Gunderson *et al.*, 2010).

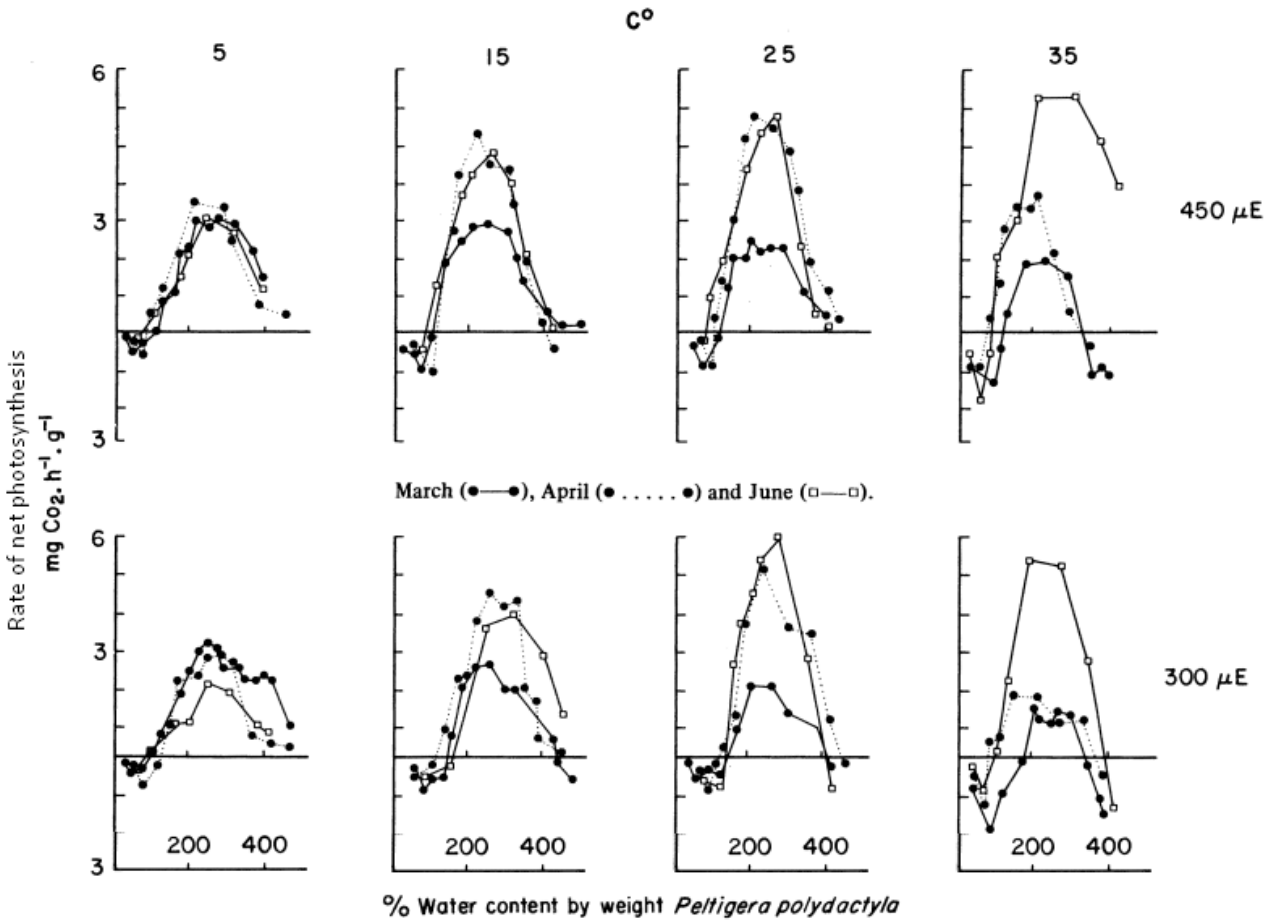

Figure 1.5.1: Net photosynthetic rate of *Peltigera polydactyla*, at 300 and 450 µE m-2 s-1 light intensities, and 5˚, 15˚, 25˚ and 35˚C. Water content is expressed as a percentage of oven dry weight. Mean rates are given from thalli collected at different seasons through the year. From Kershaw (1977) - Figure 4.

1.6 Ecological response of lichens to climate change

1.6.1 Evidence of past climate change affecting lichen ecology

Climatic factors are generally considered to be an important factor determining species ranges. Climatic conditions determine the global distribution of biomes and ecosystems with which certain species are associated (Laurent *et al.*, 2004). Past and current tundra biome distributions have been modelled successfully utilising data on average monthly temperature, light and precipitation and their effects on accumulated growing season temperature (expressed as growing degree days above 0˚C) and soil moisture (Kaplan *et al.*, 2003).

As lichens make up an important part of the biomass of arctic tundra biomes one would expect to be able to also predict potential changes in the distribution of certain lichen species according to similar factors. Bioclimatic affinity grouping, where combinations of certain key species can be mapped according to climatic parameters such as: monthly precipitation, wet-day frequency, diurnal temperature range and sunshine hours; have been used to reconstruct current species ranges with 74% mean accuracy in Europe (Laurent *et al.*, 2004). In a simplistic model one would expect with a global rise in temperature to have a simple northward shift of the existing biome boundaries and hence a northward migration of the species ranges within that biome (Skre *et al.*, 2002). But there are barriers to species migration where species attributes (e.g. low dispersal rate) or abiotic factors (e.g. land history) can retard migration (Skre *et al.*, 2002). So far this review has provided evidence for the direct effect of climatic factors on the physiological processes of lichens and some ways in which this may effect the population and the distribution of lichen species.

Climate not only affects a lichen species' distribution through direct physiological change. Many articles have highlighted the importance of competition with higher plants. Lichens in polar regions tend to be extremophiles and evolutionarily have sacrificed fast growth for the constitutive production of carbon-based secondary compounds (CBSC) (Nybakken *et al.*, 2009). This energetically expensive investment means that lichens can grow in areas where higher plants may not (Kappen, 2000; Shaver and Chapin, 1991). Climate scenarios models predict a warmer future climate that is more favourable to plant growth. Higher temperatures in the arctic tundra are hypothesized to lead to greater biomass of tundra shrub and other higher-plants associated with the biome (Olofsson *et al.*, 2009).

Global warming is thought to have contributed to increases in Arctic shrub biomass over the last century (Hollister *et al.*, 2005; Walker *et al.*, 2006). The increase in plant biomass, specifically leaf cover and canopy height, negatively affects lichen biomass accumulation, possibly through light

exclusion or nutrient competition (Cornelissen *et al.*, 2001; Hollister *et al.*, 2005). Arctic regions are also anticipated to experience more precipitation, potentially changing the hydrology of the biome. Additional moisture availability has been demonstrated to significantly increase *Sphagnum spp.* growth in arctic peat-land the accumulation of moss biomass was linked directly to a significant reduction in cryptogam species richness and lichen abundance; see Figure 1.6.1 (Lang *et al.*, 2009). In the absence of competition with vascular plants in tundra ecosystems lichens have been shown to benefit from warmer climate in the arctic and actually show greater biomass accumulation suggesting the importance of the competitive effect in determining lichen survivorship (Biasi *et al.*, 2008; Cornelissen *et al.*, 2001).

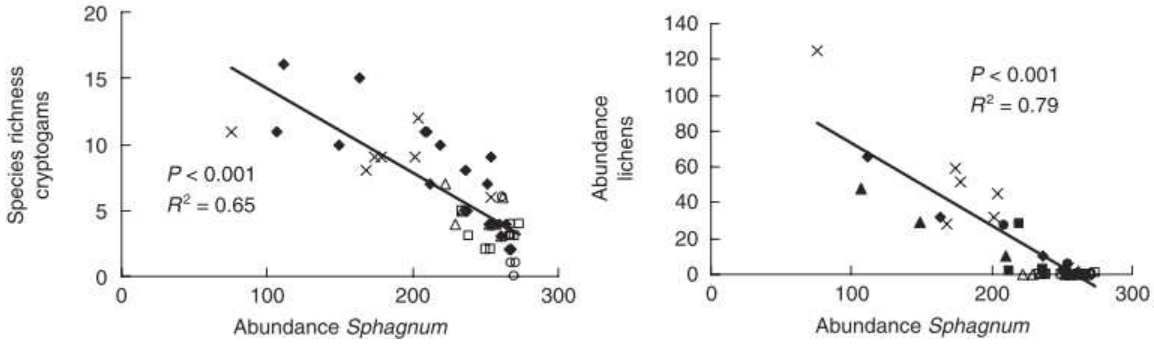

Figure 1.6.1: Scatter plot illustrating correlation between lichen abundance, and cryptogam species richness, against *Sphagnum* moss abundance. Abundance is a value of number of individuals on a plot and does not completely equate to above ground biomass. Linear regression analysis indicates that both relationships are significant (P<0.001). From Lang (2009) – Figure 3.

A similar scenario where lichens are out-competed by vascular plants occurs with the introduction of extra nitrogen (N) to an environment (Chapin *et al.*, 1995; Cornelissen *et al.*, 2001; Jagerbrand *et al.*, 2009). Warming of the Arctic would increase the rate of soil activity and the rate of turn-over of decomposition; this would ultimately lead to the availability of additional nutrients, such as N (Nadelhoffer *et al.*, 1991; Petchey *et al.*, 1999; Shaver *et al.*, 2001; Van Cleve *et al.*, 1990). Experimental N deposition resulted in an expansion of *Betula nana* shrub biomass, leading to general decrease in biodiversity in tundra shrub microcosms; including reduction in lichen biomass and species richness (Shaver *et al.*, 2001). Increased nitrogen availability does increase lichen-thallus growth- rate and photosynthetic capacity but in areas where plants are present shading, induced by competition, counteracts this affect (Nybakken *et al.*, 2009; Palmqvist and Dahlman, 2006).

A warmer climate will also affect herbivores that specialise in consuming lichens. Invertebrate herbivores such as Collembola and mites have been documented feeding of arctic lichen (Hodkinson *et al.*, 2004; Hodkinson *et al.*, 1996). Higher temperatures have been shown to be detrimental to arctic invertebrate populations (Coulson *et al.*, 1996). The effect of invertebrate grazing on terricolous lichen biomass in the arctic tundra has not been, to the best of my knowledge, reviewed in the literature so whether loss of invertebrates will have a significant effect or not is not known. However exclusion of invertebrate grazing in a deciduous forest habitat increased the survival of juvenile *L. pulmonaria* thalli (Asplund and Gauslaa, 2008). Furthermore the vertical distribution epiphytic *Lobaria* species is significantly affected by the grazing patterns of gastropods; which could possibly translate into a similar horizontal distribution effect where low temperatures produce refugia from invertebrate grazing; a warmer climate could lead to the loss of these hypothetical refugia and affect lichen growth dynamics (Asplund *et al.*, 2010). Loss of lichen-eating invertebrates due to climate change may reduce dispersal capabilities of some lichens; viable spores of *Xanthoria parietina* have been found in the faecal pellets of lichenivorous mites, *Trhypochtonius tectorum* and *Trichoribates trimaculatus*, in an *ex situ* study suggesting a possible dispersal mechanism of both the photobiont and the mycobiont (Meier *et al.*, 2002). A zoochorous dispersal mechanism could be susceptible to climate change.

Forest fires have a significant effect on lichen productivity in Canada; disturbance events disrupt thallus growth and favours faster-growing annual plants (Joly *et al.*, 2009). Global warming would potentially lead to a greater number and intensity of forest fires in Arctic environments. Lichens have a higher albedo, when compared to shrubby taxa, and the replacement of lichen tundra with herbaceaous biomass may lead to higher temperatures and more forest fires (Chapin *et al.*, 2005; Higuera *et al.*, 2008). Fire also increases nitrogen availability which has already been shown to significantly and negatively affect lichen species richness and productivity.

1.6.2 Future climate change

The Arctic is currently the warmest it has been for the last 400 years (Overpeck *et al.*, 1997). The rising temperature is thought to be mostly due to anthropological forcing and future climate change scenarios modelled on the IPCC emissions scenarios are thought to be accurate (Overpeck *et al.*, 1997; Serreze *et al.*, 2000). General global predictions suggest that higher latitudes will be affected by climate forcing more than lower latitude areas, due to a feed back mechanism where loss of sea ice and snow cover decreases albedo and causes further warming (Serreze *et al*., 2000). Annual

mean ground surface temperatures in the Northern hemisphere have been predicted to rise by as much as 7K (Kelvin) but with a greater increase in winter temperatures than summer ones (Eliseev *et al.*, 2009). Soil warming would result in greater soil activity and potentially stimulated respiration levels will induce a rise in the CO_2 concentration available for photosynthesis and lead to a rise in lichen NP (Coxson and Wilson, 2004). A shorter cold period will be translated into less snow cover and a longer spring and autumn growth period (Eliseev *et al.*, 2009; Serreze *et al.*, 2000). These conditions have been simulated and a longer autumn and spring wet active period along with an increase in temperature are hypothesised to results in enhanced lichen photosynthetic activity and biomass accumulation (Cabrajic, 2009). However where light is limiting, such as dense forests or were vascular plant biomass causes shading, higher temperatures and longer wet periods will cause greater respiration rates and result in a more negative carbon balance and retard lichen biomass accumulation (Hollister *et al.*, 2005; Olofsson *et al.*, 2009; Cabrajic, 2009). Drier summer conditions in the Arctic will further reduce wet time in the most important growing season for many Arctic lichens and may lead to more negative carbon budgets (Cabrijic, 2009; Uchida *et al.*, 2006). This change in lichen NP could lead to losses in lichen species richness in areas which contain large proportions of total lichen species, as well as have a significant effect on tundra C balance.

Snow cover in thought to beneficial to lichens during deep Arctic winter as it protects lichen thalli from extremely low temperatures and reduces damage caused by diurnal freeze-thaw cycles (Bjerke, 2009; Kappen *et al.*, 1995; Korner, 1999). Predicted loss of winter snow may result cold damage (Showman, 1979), though unlikely in psychrophilic Arctic lichens, and exposure to prolonged freeze-thaw events which can cause leaching of carbon compounds and cause "resaturation-respiration" events which would reduce winter net production (Kappen *et al.*, 1995; Tearle, 1987). Contrarily snow-cover has been shown to cause reductions in lichen biomass accumulation due to snow reducing the light intensity reaching hydrated thalli (Benedict, 1990). Snow-cover may also slow warming from deep winter temperatures and delay the onset of spring-growth and reduce net productivity during an important part of the year (Pannewitz *et al.*, 2003). Snow-cover is evidently an important but complex component in determining lichen productivity, a wide range of factors need to be considered, see Figure 1.6.2, making prediction of response of lichens to future loss of snow-cover difficult. A good review of the effects of snow-cover can be found in Kappen *et al.* (1995).

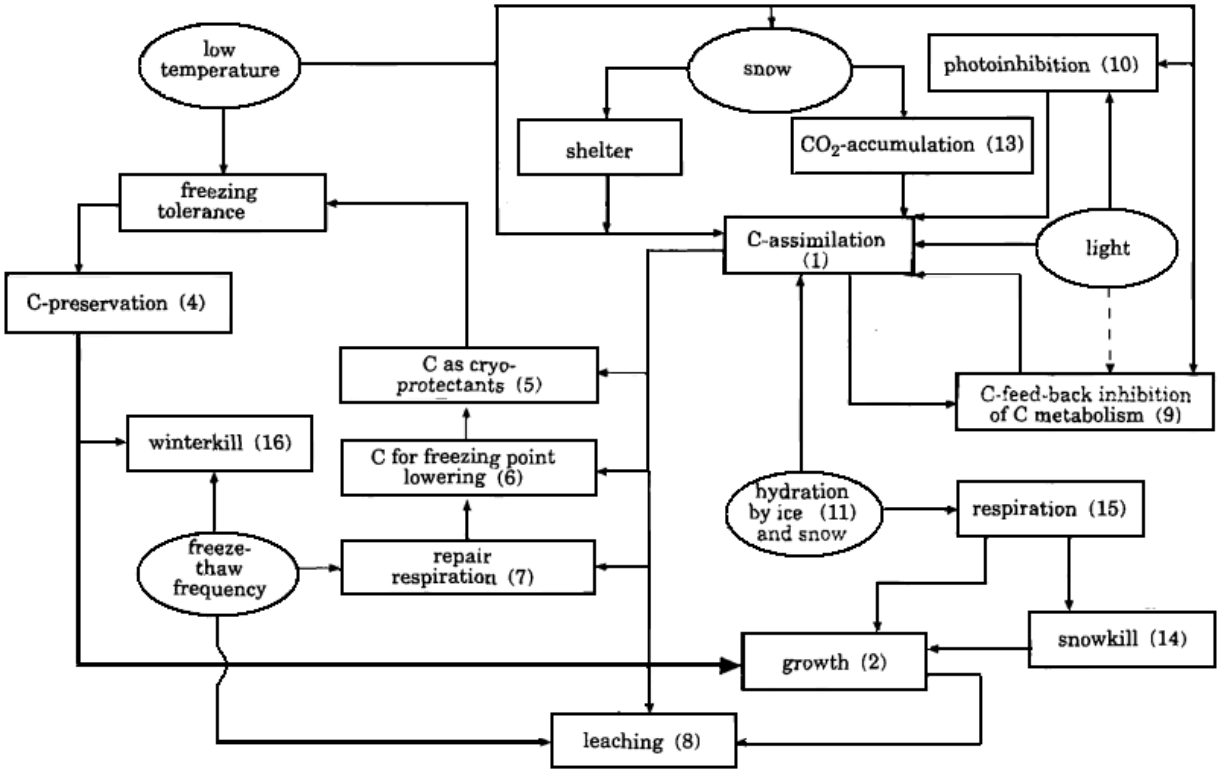

Figure 1.6.2: Concept flow-chart illustrating the complex series of interactions that form a lichens response to snow-cover, specifically relating to carbon acquisition. Numbers are for reference with the original paper on the subject. Note that in many cases this flow chart is true for carbon acquisition at any time of the year in a tundra environment. From Kappen *et al.* (1995) – Figure 7.

In combination with climate change through the "green-house effect", anthropogenic climate forcing factors such as aerosols and aerosol precursors, especially from jet engines, have also been shown to cause a phenomenon known as "global dimming" (Wild, 2009). "Global dimming" is the process by which particles in the upper atmosphere scatter/absorb incoming solar radiation and cause a reduction in the amount of solar radiation that reaches the ground surface (Wild, 2009). Future scenarios suggest that developing countries may increase their output of particles which contribute to further "global dimming" (Wild, 2009) which may result in a loss of PAR available for lichen photosynthesis. Lower PAR combined with warmer wetter climate could result in a lower rate of biomass accumulation in Arctic lichen (Cabrajic, 2009).

1.6.3 Non-climate related factors affecting the ecology of sub-Arctic lichens

There are other factors that affect lichen ecology in the arctic and sub-arctic that are not related to climate. Hauck (2009) suggests that loss of terricolous lichen species in the Netherlands and North Germany over the 20th Century may be related to loss of nutrient-poor soils found in heathland habitats through their conversion to arable land. Although the conversion of arctic tundra heath into arable land is unlikely, the hypothesis lends credence to hypothesise that increased N and other nutrient availability in tundra biomes will lead to a decline in lichen biomass and potentially lichen species richness (Cornelissen *et al.*, 2001; Grellmann, 2002). This is less likely to affect saxicolous lichen species as they tend to experience less competition with vascular plants and the substrate upon which they grow is unsuitable for fast-growing tracheophytes (Hauck, 2009).

Reindeer graze on lichens as winter forage which decreases lichen biomass (Olofsson *et al.*, 2009). Tommervik *et al.* (2009) suggests that the relative reduction of lichen biomass, in two provinces in Norway, is related to the population of reindeer in that area and has found a significant correlation between the two. Reindeer grazing removes the upper layer of mat-forming lichen, which is often the part of a thallus that contains the highest concentration of photobionts and results in a significant reduction of the Chl *a* content of grazed thalli (Gaio-Oliviera *et al.*, 2006). Reduced Chl *a* content does lead to a reduction of photosynthetic capacity and the capability of lichen to re-grow after grazing (Dahlman and Palmqvist, 2003; Gaio-Oliveira *et al.*, 2006). However, newly established lichen stands in North Swedish pine forests, where reindeer are known grazers, increased their ground cover and thallus population under a heavy grazing regime (Roturier and Bergsten, 2009). Further evidence exists suggesting that whilst ungulate grazing affects lichen species biomass, and tundra community structure, it does not, in short term experiments, contribute to the loss of lichen species richness (Holt *et al.*, 2008). Fragmentation of mat-forming lichen by grazing, breaking up the boundary layer which reduces water loss form mat-forming organisms (Zotz *et al.*, 2000), has not affected length of active wet time in past experiments (Gaio-Oliviera *et al.*, 2006). In combination with environmental effects vertebrate grazing has the potential to cause significant effects on lichen productivity and could affect non-vascular NP.

Some lichen species are considered to be susceptible to damage from environmental pollution. Sulphurous compounds which enter the atmosphere through industry and vehicle emissions have been shown to cause degradation of chlorophyll, interfere with the exchange of nutrients between lichen bionts and reduce protein synthesis rates (Conti and Cecchetti, 2001). The number of morphological aberrations in thalli of *L. muralis* positively correlates with closeness to city centres, the trend is thought to be affiliated with exposure to sulphur dioxide (SO_2) (Seaward, 1976).

Pollution is known to cause a reduction in the photobiont : mycobiont ration of a thallus, resulting in reduced productivity (Seaward, 1976). Atmospheric pollution is thought to be a major factor in the decline in lichen species in Europe and is a potential threat to future lichen biodiversity (Aptroot and van Herk, 2007; Hauck, 2009).

1.7 Aims of the present study

The following studies are an extensive investigation of the effect of changing climatic factors on the rates of net photosynthesis (NP) and dark respiration (DR) in lichen species that are an important component of Arctic tundra ecosystems. The lichens inhabit different niches, within the tundra ecosystem, and cover a range of morphological types (*see Chapter 2*). These investigations have focused on metabolic activity as a proxy for the performance of lichens under set environmental conditions; previous experiments have shown that laboratory studies of photosynthesis and respiration, of lichens, are good predictors of how individuals behave in their natural environment (Cabrajic, 2009; Lange *et al.*, 2001; Lange and Green, 1996; Rieter *et al*., 2008). To have complete control of the climatic variables the experiments were conducted in a laboratory environment. The aims of the two main studies were as follows:

The effect of short-term (over the period of an hour after long-term storage at an arctic-tundra mid-summer temperature) changes in air temperature and PAR intensity, on the rate of NP and DR of sample thalli, has been tested in four lichen species: *Nephroma arcticum*, *Cladina rangiferina*, *C. stellaris* and *C. mitis*. The experiment had two main functions: firstly to find the optimal light intensity and air temperature of NP, for all four species and secondly to investigate the presence of a co-variant effect of light intensity and temperature on photosynthetic rate; as hypothesised in Lange (2002). The experiment also allowed a working methodology, for the measurement of NP and DR of lichen thalli using an oxygen electrode, to be assembled. It is hypothesised that if statistically, temperature and light intensity affect all species equally, then lichens can effectively be considered as one type of organism within the landscape, and be modelled as such in primary productivity estimates.

The long-term (6 day-long exposure to mean average temperatures of 5˚, 10˚ & 15˚C) affect of temperature on NP and DR was also studied. *C. mitis*, *C. stellaris* and *N. arcticum* were examined. The speed at which lichen thalli acclimatised to the temperature regimes was analysed as well as any differences in response to air temperature patterns over the course of six day acclimation period. The results here could provide important information as to how to correctly store lichen sample *ex situ* as well as predict how phenotypically plastic different species are.

Laboratory experiments are crucial in the understanding the mechanisms behind the response of any species to climate change (Lange, 2002; Lange *et al.*, 1998; Lepetz *et al.*, 2009). The analysis from the experiments reported here will aid in the understanding of results from future experiments in the significance of non-vascular production in the tundra carbon cycle, the ecological response of

reindeer forage to a changing climate and potentially how future climate predictions will affect cryptogam species richness. The studies have also considered whether there are any patterns in the response of species to environmental factors and species morphology or genus. Evidence of similarities between species with similar morphologies, or of the same genus, would help in efforts to model annual production in lichens and how it will change in the future (Cabrajic, 2009; Lange, 2002). The comparisons that have been made between species will also help resolve whether it is prudent to use a single species as a model upon which to base the response of all lichen species from a species environment.

1.8 Dissertation outline

The next Chapter details the importance, distribution and niche habitat of the four species of lichens studied in this report, the Chapter also outlines the method used to store the lichen samples and how thalli were prepared before each measurement.

Chapter 3 records the preliminary experiments that were required in order formulate a working method, by which the rate of NP and DR of individual lichen thalli could be measured, using the oxygen electrode apparatus.

Chapters 4 & 5 are reports of the two experiments performed, outlined in the aims, testing the effect of environmental factors on metabolic activity in the four lichen species studied.

In Chapter 6 the results from Chapters 4 & 5 are analysed together, and the potential impact of the data, in regards to current literature on the ecology of sub-Arctic lichens, is discussed.

Chapter 2

Samples

2.1 Lichen species ecology

The present study has focused on four species of lichen which are common in sub-Arctic tundra-heath/boreal ecosystems. The species were chosen as they comprise a significant proportion of biomass within the ecosystem, they also provide a range of morphological types. The use of crustose lichen species, which occur on rock substrate, is not ideal when applying the methodology employed in these studies, as they do not fit inside the electrode chamber. However species, which exist directly upon rock as a substrate, do not make up a significant proportion of biomass in arctic tundra-heath ecosystems, and are not included in studies of the primary productivity of lichens within this specific ecosystem (Lange *et al.*, 1998; Walker and Walker, 1996).

Nephroma arcticum (L.) is a foliose green algal lichen species which also form a symbiosis with nitrogen fixing *Nostoc spp.* cyanobacteria; potentially making it an important part of the N-cycle (Cornelissen *et al.*, 2007). The species has a very wide circumpolar distribution and is found at high altitude in more Southern areas, within these global distributions *N. arcticum* typically exists in the heath ecosystems of the northern boreal regions. *N. arcticum* thalli predominate in the shelter of larger plants in subarctic woodland, however they are found on cooler, shaded North facing slopes in more open heath areas, where they compose most of the ground layer of vegetation in *'Empetrum* type' heath ecosystems; which are indicative of relatively base nutrient-poor soils (Svensson and Callaghan, 1988). *N. arcticum* presence, within heath habitats, is positively associated with presence of shrubs, such as *Vaccinium myrtillus*, and pleurocarpic mosses, such as *Hylocomium splendens*, and negatively associated with the presence of the lichen *Peltigera apthora* (Svensson and Callaghan, 1988). There is evidence of the associations between *N. arcticum* and plants species in Figure 2.1.1 where *N. arcticum* was most abundant on the North-facing woodland habitat which contained a high ground surface coverage of *H. splendens* and *V. myrtillus* but a low number of *P. aphthora*. On the South facing slope *P. apthora* was more dominant, and although there was a high ground-coverage by *H. splendens* and *V. myrtillus*, there were no thalli of *N. arcticum* present. *N. arcticum* is the most shade tolerant of the four species analysed in this report, and is more closely associated with higher plants than the *Cladina* species, it was expected that, as shaded thalli are less exposed to light radiation, the species will exhibit the greatest level of photoinhibition in high light scenarios. The shaded mesic environment is colder through longer periods of the year than the exposed tundra environment, in which the three *Cladina* species analysed in this study are found, it was expected that

N. arcticum would be the least heat tolerant before being given a chance to acclimatise (Sonesson *et al.*, 1992). Although the species is found in the most sheltered environment, of the four species studied, it has been shown to exhibit a high level of seasonal photosynthetic plasticity, and changes its optimum temperature of photosynthesis through the year (Sonesson *et al.*, 1992). It is expected that the species will exhibit rapid acclimatisation and a high level of photosynthetic plasticity, during the 'long-term exposure to temperature' experiment reviewed in Chapter 5.

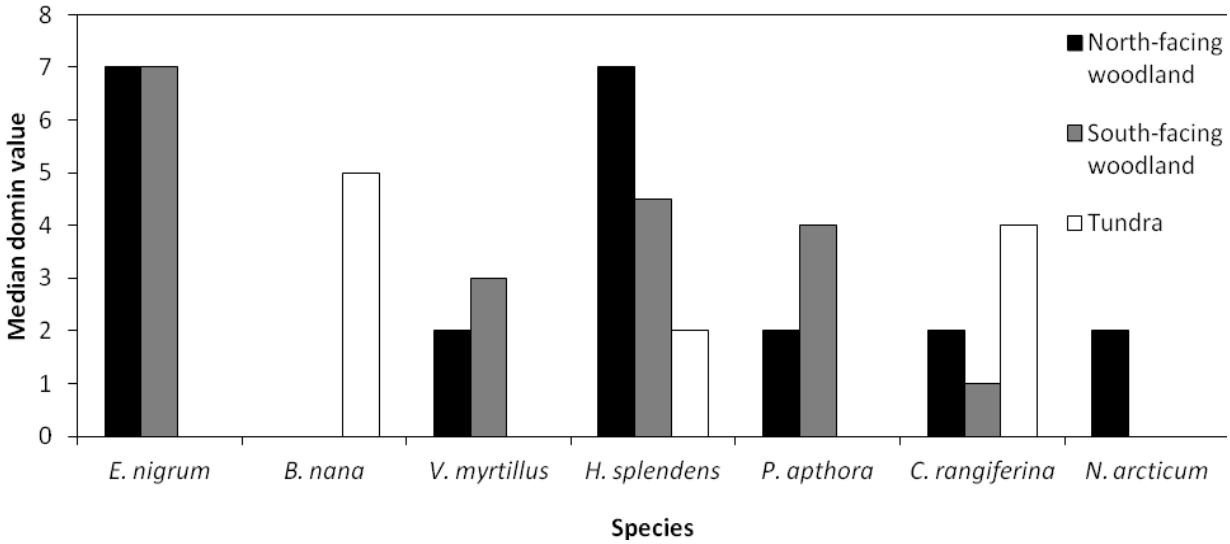

Figure 2.1.1: Ground coverage of 2 m x 2 m quadrats by species in three different habitats within the Northern boreal forest region in Abisko, Sweden. Species illustrated in this Figure are some of the species considered to be important indicator species of each habitat and two of the lichen species studied in this report. Median domin values indicate cover abundances from seven replicate quadrats, values are classified in Table 2.1.1. Baxter and Henri, Unpublished.

Table 2.1.1: Assigned values for the domin scale ustilised in Figure 2.1.1.

Domin Value	Percentage ground cover	Further defined as
1		one or a few individuals
2	< 4 %	scattered indivduals
3		frequent
4	4 ≤ x ≤ 10 %	
5	10 < x ≤ 25 %	
6	25 < x ≤ 33 %	
7	33 < x ≤ 50 %	

The *Cladina* species, analysed in the studies presented in this report, are all fruiticose lichen species with green-algal photobionts, which form dense mats of thalli. Collectively the three *Cladina* species analysed are known as reindeer lichens they have a fully circumpolar spread throughout the Northern hemisphere. Reindeer lichens are relatively shade, and moisture, intolerant, when compared to *N.arcticum* and other circumpolar lichen species, and in the Fennoscandian region (where these samples have been collected from) are found in the drier, exposed tundra habitats. Figure 2.1.1 illustrates that *C. rangiferina* (L.) Weber ex Wigg, one of the species analysed in the studies in this report, had a highest biomass in the tundra habitat, where there was a reduced vascular plant presence, with no *Empetrum nigrum* or *V. myrtillus* although there was some *Betula nana* present. Reduced vascular plant presence means less leaf canopy and hence less shading of thalli.

C. rangiferina is the most shade and moisture tolerant of the three *Cladina* species analysed and grows best in sheltered less xeric habitats than the other two, *C. stellaris* (Opiz) Brodo is the most xerophytic of the reindeer lichens growing in the most exposed tundra environments and *C. mitis* (Sand) is found predominantly in environments which are a middle-ground between the other two *Cladina* species (Lechowicz and Adams, 1974; Ringius and Sims, 1997; Tegler and Kershaw, 1980). *C. mitis* presence is indicative of and early successional stage within and environment and is found in habitats that exhibit a higher seasonal variation in temperature and light intensity than the other reindeer lichens (Kershaw, 1977). As an adaptation to the highly variable environmental conditions that it inhabits *C. mitis* thalli have been shown to exhibit a greater level of seasonal photosynthetic plasticity than *C. rangiferina* (Lechowicz & Adams, 1974). *C. stellaris* has not only been shown to exhibit significant variation of photosynthetic rate and optimal conditions of photosynthesis over the changing seasons, but also exhibits photosynthetic differences between ecotypes, ridge-top and ridge-bottom thalli, that are concluded to be due to phenotypic plasticity, as opposed to genetic differences (Kershaw *et al.*, 1983). Presence of seasonal photosynthetic plasticity has only been shown in *Cladina* species from the North American continent, and not from Fennoscandian populations at it has in *N. arcticum* thalli. Due to the high variability, in the response of lichens to a changing environment, thalli from the European continent should be analysed to see if the response to climatic factors differs between the two populations. These three *Cladina* species form the most important component of reindeer winter forage globally and could cause significant damage to *Rangifer* populations if the lichens were to be lost from their diet.

Species nomenclature follows Brodo *et al*. (2001).

2.2 Storage

Introduction

Lichen thalli have been shown acclimatise, i.e. to adapt, to different climate regimes, and will change their response to experimental control of external conditions according to the environment in which they have been stored (Kershaw, 1977). As described in the introduction, lichens are capable of changing their thallus physiology to adapt to different environments, such as changing chlorophyll concentration and the optimum temperature of enzymes involved in photosynthesis, which will affect the rate of gaseous exchange and reduce the reliability of any comparison experiments (*Chapter 1*). In order to create a base from which all thalli start with the same morphology and physiology, samples were placed in climate-controlled growth chambers (Fitotron growth chambers: Weiss Gallenkamp, Loughborough, Leicestershire, UK). Samples have been kept at 15˚C throughout the time course of the study (except in the experiment reported in Chapter 5 where storage air temperature varied); as summer is suggested to be the peak growth season of lichens and 15˚C was shown to be the mean mid-day summer temperature in climatological data of Abisko (Baxter, unpublished; Williams *et al.*, 2000). Light was kept at non-photoinhibiting levels of 100 µmols m^{-2} s^{-1} to ensure firstly that the samples did not experience photodamage, and secondly that samples were capable of photosynthesis so thalli did not deteriorate between measurements. It was decided that samples would not be stored frozen as although this has been done with certain experiments (Callaghan *et al.*, 2004c; Gauslaa and Solhaug, 1999; Kappen *et al.*, 1995).

After prolonged periods of desiccation, lichens are often known to experience a prolonged period of elevated respiration, known as resaturation respiration that can last up to three days *(see* Introduction). Previous to any experimentation, whenever lichen thalli had remained desiccated for longer than 5 days, i.e. when samples were first handled in the UK, samples were given at least one week of daily hydration and desiccation cycles, to reduce the effect of resaturation respiration (Rogers, 1971).

Method

In order to test the effect of prolonged dry storage on the lichens samples, the O_2 electrode apparatus was set up, as per manufacturer instructions. Ten previously unused samples of *C. rangiferina* and *N. arcticum* were submerged in distilled water for 5 min and then blotted with tissue paper, for optimal hydration. All measurements were taken at 15˚C and at 710 µmol m^{-2} s^{-1}. After measurements were taken samples were allowed to desiccate in the storage chambers. Everyday samples were hydrated in distilled water then allowed to desiccate in the storage chambers, at the

above conditions. Sample gas exchange was then remeasured a week and two weeks after first hydration.

When comparing all four species, the samples were separated as per the instructions in section 2.3. Sample metabolic rate was measured, with an O_2 electrode, at time of first hydration after prolonged dry storage and again after approximately two months of storage, under the above conditions, with periodic wet and dry cycles. Statistical analyses were perform using SPSS 15.0 for windows.

Results

One week was shown to be a sufficient amount of time to reduce the suppression of net photosynthesis, caused by resaturation respiration, in *C. rangiferina* and *N. arcticum*, as there was an increase in mean net photosynthetic rate after one week of hydration cycles but no increase in the second week (Figure 2.2.1).

An ANOVA test comparing the mean rate of net photosynthesis, for each species, at the two different times did not find any significant differences: *C. mitis* ($F_{(1\ 18)}$= 0.661, *P*= 0.427), *C. stellaris* ($F_{(1\ 18)}$= 0.31, *P*= 0.863), *C. rangiferina* ($F_{(1\ 18)}$= 0.054, *P*= 0.821) and *N. arcticum* ($F_{(1\ 18)}$= 0.002, *P*= 0.966).

Discussion

As one week of hydration/desiccation was sufficient to ameliorate the effects of prolo nged dry storage, samples were conditioned for one week prior to any further measurements. Both *C. mitis* and *C. stellaris* exhibited no increase in net photosynthesis in Figure 2.2.2, so it is assumed that the one week provided before photosynthesis measurements were undertaken, was sufficient to remove the effects of resaturation respiration on net photosynthetic rate in these species.

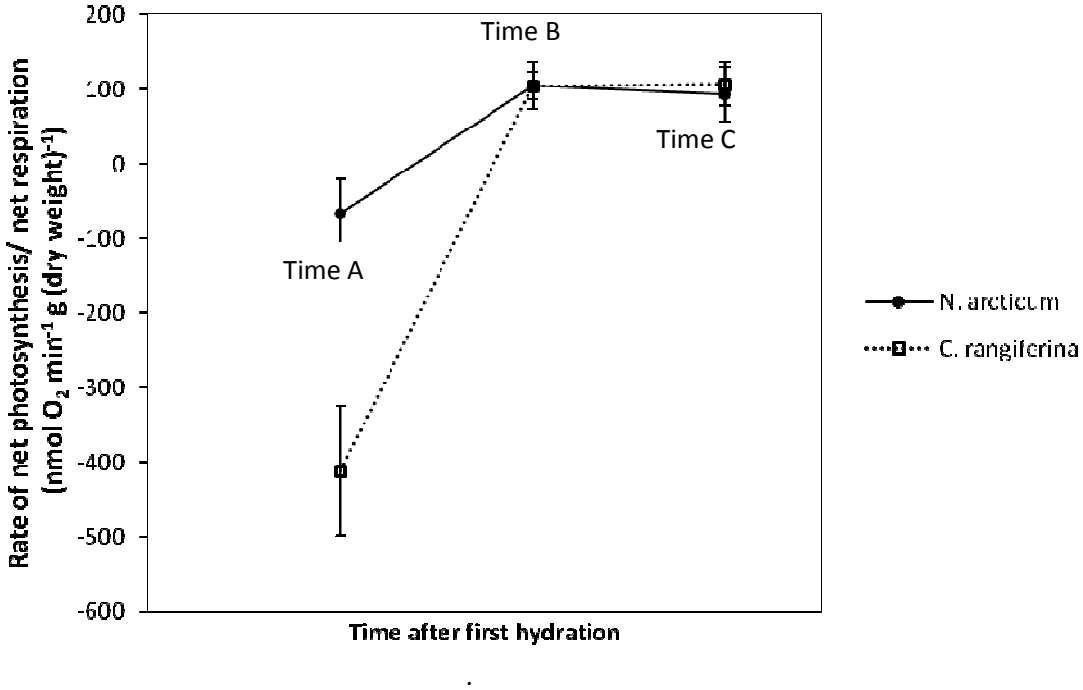

Figure 2.2.1: Mean rate of net photosynthesis/ respiration immediately after samples were first hydrated (Time A), after a week (Time B) and after two weeks (Time C) of daily hydration/desiccation cycles for two species of lichens. Error bars indicate +/- one s.e. from the mean (n=10).

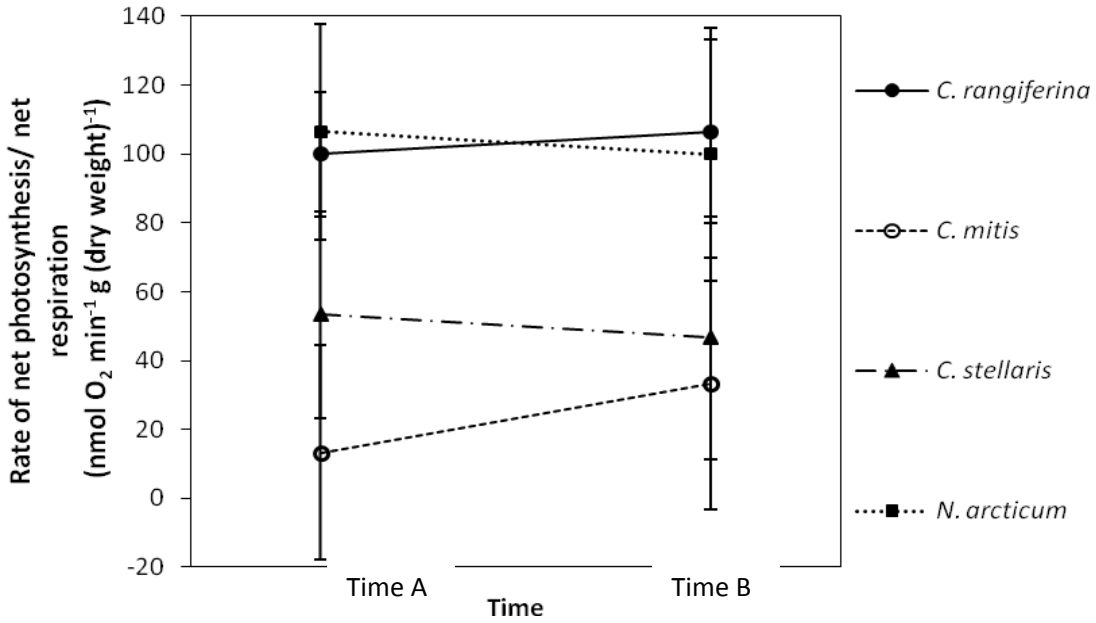

Figure 2.2.2: Mean net rates of photosynthesis of all four species analysed in this report at time of first measurement (Time A) and two months later (Time B). Error bars indicate +/- one s.e. and N= 10 for all species (apart from *C. rangiferina* where N= 7).

2.3 Creating samples from lichen thalli

In order to create a standard sample size, four of five thalli of each species were placed within a desiccation chamber. Prior to desiccation samples were cleaned of extraneous matter. The desiccation chamber was an enclosed glass container with a layer of mesh, upon which thalli could be placed, suspended over anhydrous silica. The thalli were desiccated until they were at equilibrium with the air in the chamber. Samples of 0.15+/- 0.005 g where then separated out using electronic scales; when creating the samples for the fruiticose species only the tips of the thalli were used to minimise differences in chlorophyll content and wet weight. Dry weights were obtained by oven drying, 24 hours at 105˚C, after samples were no longer going to be used. The dry weight of each lichen thallus was used to calculate the rate of NP and DR per gram of dry weight using Equation 1:

Equation 1:

$$rate\ of\ metabolism\ per\ gram\ dry\ weight = \left(\frac{1}{dry\ weight\ of\ sample\ (g)}\right) * rate\ of\ metabolic\ process$$

Samples were created to minimise wastage of stock material and reduce variation within the data resulting from using multiple thalli. Samples were wetted at regular intervals to allow metabolic activity and to stop thalli degeneration, but not stored in a hydrated state, as wet storage in lichens causes oxidative damage as it results in extra production of reactive oxygen species, which results in depressed photosynthetic rates (Minibayeva and Beckett, 2001). Preliminary experiments showed that the NP of lichens did not significantly deteriorate within the two months of the longest experimental period; statistical analysis (ANOVA test; see Figure 2.2.2) revealed no significant differences between the mean photosynthetic rates of each species and the two times (Figure 2.2.2). Thus reuse of samples is justified.

Chapter 3

Methodological development

3.1 The importance of metabolic rate data as an ecological tool

In this experiment lichen performance has been measured, as it has in previous studies, in terms of metabolic activity. Respiration and photosynthesis rates in lichens are often measured in laboratory experiments to provide qualitative values of the response of lichen species to a change in environmental conditions (Benedict, 1990; Kappen et al., 1995; Lange and Green, 1996; etc.). It is important to obtain quantitative data as they allow statistical comparisons between different species responses as well as response of the same species under different conditions; measuring metabolic activity, in terms of uptake/ release of moles (mols) or grams (normally mg) of carbon or oxygen, provides data that can be used to discover whether the response to variable environmental conditions can be predicted by knowing about the genetics or ecology of the species (Ino, 1985).

Metabolic activity rates are an important ecological tool: they can be used to estimate net productivity and quantify the contribution to the ecosystem carbon cycle (Biasi *et al.*, 2008; Jonsson *et al.*, 2008; Lange, 2003a; Lange, 2003b; Lange *et al.*, 1998; Lepetz *et al.*, 2009; Uchida *et al.*, 2006). Net photosynthetic rates have also been shown to be a good indicator of growth rate of thalli, which are important in determining the reproductive success and survivability of lichen species (Benedict, 1990; Bjerke *et al.*, 2005a; Cabrajic, 2009; Gauslaa, 2006). A study has shown that the rate of biomass accumulation of a *Cladina stellaris* thallus is directly correlated to the net photosynthetic rate through the year (Nash, 1996).

The rate of metabolic activity in lichens has been shown to change according to certain external, environmental factors (*see Chapter 1*). The habitat within which a lichen species exists can be inferred according to how the rate of photosynthesis, and respiration, of a thallus responds to the environmental factors that affect metabolic activity (Aubert *et al.*, 2007; Jonsson *et al.*, 2008; Liden *et al.*, 2010; Reiter *et al.*, 2008). For example, lichens that are capable of photosynthesising at higher light intensities and show greater ability to reduce photoinhibition levels will inhabit areas where they are more exposed to light than a species/ecotype which has a lower optimum light intensity; i.e. light intensity at which the greatest levels of net photosynthesis occur (Sonesson, 1989). Furthermore the response of a sample of lichen thalli to experimentally-induced external environment conditions can be used to anticipate how a species will respond to predicted future climate change and allow some comprehension of the mechanism which evokes the response (Lange *et al.*, 2001; Lepetz *et al.*, 2009; Reiter *et al.*, 2008; Schipperges and Gehrke, 1996).

3.2 Measurement of the metabolic rates of lichen thalli

3.2.1 Common apparatus used in the field of lichenology

In the literature reviewed, the response of lichen metabolic rates to changes in the environment has been measured in two ways: the most common, and long-running practice, has been to measure changes in gas concentrations in air surrounding a lichen thallus; the second is by measuring chlorophyll fluorescence. The latter choice has been shown to be an effective method of measuring photosynthetic activity in higher plants (Genty *et al.*, 1989). However there are problems with such chlorophyll fluorescence measurements: Firstly they do not give any indication of respiration rates of a sample which is an important part of calculating net photosynthesis. This is an important problem as thallus respiration is not constant, is influenced by climatic variables, and can often be a significant suppressor of net photosynthesis; *see introduction*. Secondly it has not, to my knowledge, been categorically proven that measurements of electron-chain activity, recorded using fluorescence methodology, and photosynthetic rates, measured through gas-exchange methods, have a simple linear (or curvilinear) relationship; as they do in higher plants. Green *et al.*, (1998) have concluded that the relationship is not simple, with the relationship between the two varying at different external temperatures, and that results produced should be backed up with extensive gas-exchange measurements. In the species *Cladonia impexa* and *Collema flaccidium* the relationship between chlorophyll fluorescence and carbon fixation rate has been shown to change according to the relative humidity at which the measurements are taken, further casting doubt on the validity of results using this method; especially when comparing fluorescence and carbon assimilation (Lange, 2002; Sigfridsson, 1980). Due to the above issues it was decided that attempting to measure fluorescence in these studies would be time consuming and detract from efforts to meet the aims set out in the introduction.

3.2.2 The IRGA apparatus – preliminary experimentation

Introduction

Mostly, photosynthetic rates in lichens have been measured with an Infra-Red Gas Analyser (IRGA) (e.g. Lange, 2002). The apparatus is used to measure the difference in carbon dioxide (CO_2) concentration in the air entering a chamber containing a lichen thallus and the concentration of the air leaving it. It is assumed that any difference in the two concentrations is due to uptake/loss of CO_2 by the thallus; therefore, photosynthesis can be measured in terms of the rate of CO_2 uptake. Gas concentration measurement methods are advantageous as they can be used to measure both net

photosynthesis and dark respiration which, as both processes vary with fluctuating environmental conditions (*see introduction*), are both important in discerning the exact manner in which net photosynthetic rate of a lichen thallus is affected by climatic factors.

Method

An LI-6400 Portable Photosynthesis System (LI-COR Environmental, Cambridge, UK) was used to test the efficacy of the IRGA apparatus for the analysis of the lichen samples. To test whether the IRGA was capable of measuring gas-exchange at the minute levels required a simple test was performed.

Initially the IRGA apparatus was used to measure the difference in the CO_2 concentration of the in- and out-flow atmospheres without any living tissue in the sample chamber. Importantly, the apparatus had been freshly calibrated, so as to ensure that it was working maximally. Before any measurement took place the chamber was cleaned with anti-bacterial wipes to ensure no living tissue could interfere with the apparatus' accuracy. The system flow was set at 0.4 l min^{-1}, the temperature maintained at 15 ^{o}C, the in-flow CO_2 concentration at 330-350 ppm, and the light intensity at 500 µmol m^{-2} s^{-1}; used previously for the measurement of the gas-exchange of sub-Arctic lichens (Groulx, 1987;Sonesson, 1992). The test was run for 10 min, with the apparatus recording the rate of gas-exchange every 30 sec; providing a total of 19 points.

Subsequently, a 0.5 g sample of *N. Arcticum*, which had been hydrated and allowed to desiccate slowly for 24 hr, was hydrated in distilled water then blotted dry; so as to optimally hydrated (Groulx, 1987). The sample was then placed to the IRGA sample chamber, and the apparatus ran for a further 10 min, under the conditions described above.

Statistical analysis was performed using SPSS 15.0.

Results

Preliminary experimentation revealed that the IRGA had inherent levels of variability of CO_2 concentration measurement higher than the actual rate of thallus loss/uptake, and the addition of a 0.5 g sample of *N. arcticum* made little difference to the mean rate of change of CO_2 concentration; an ANOVA test found the difference in mean rates of change of CO_2 concentration within the sample chamber, while the thalli were within the chamber and when it was not, not to be significant (Figure 3.2.1). Figure 3.2.2 illustrates that the range of rate values obtained by the IRGA under constant

environmental conditions, 1.5 µmol m^{-2} min^{-1} with the sample in the chamber and 4 µmol m^{-2} min^{-1} without, was many times larger than many of the results presented later.

The IRGA results also had a large proportion of anomalous data points when compared to the analysis by oxygen electrode; Figure 3.1.2 presents two significant outliers within 10 minutes of measurement for both set as results. The higher rate of anomalous points suggested a high rate of inaccuracy inherent in the IRGA apparatus.

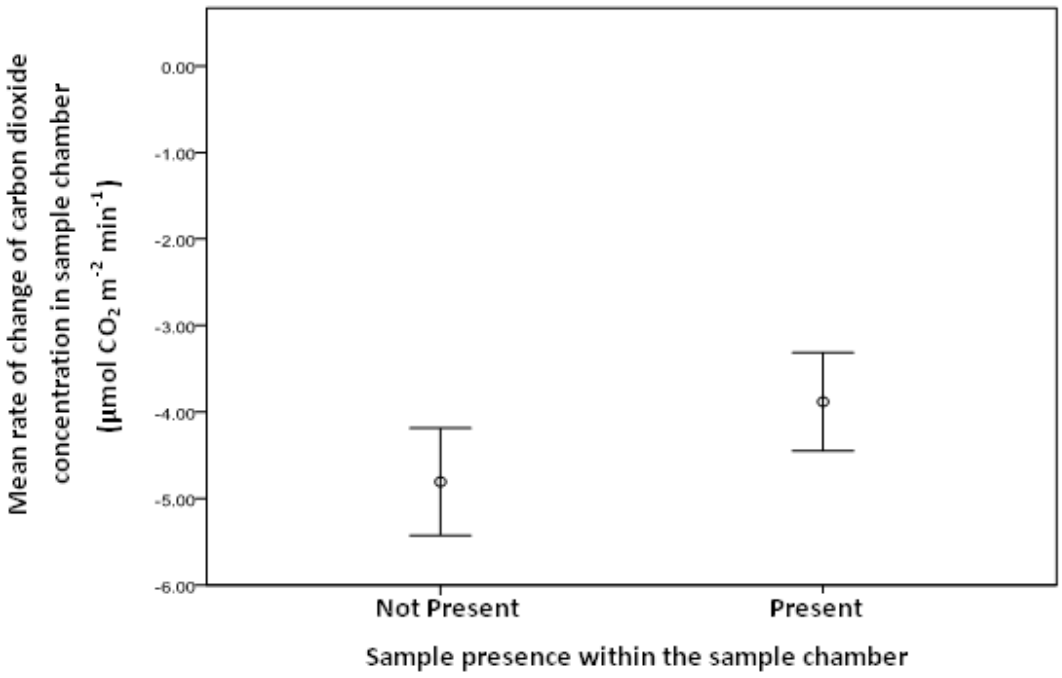

Figure 3.2.1: Mean rate of change of CO_2 concentration within the IRGA sample chamber over the course of 10 minutes. Rate values were obtained once per minute for every minute measurement took place. Results of the ANOVA comparison of the two mean values did not indicate a significant difference (F(1 37)= 1.213, *P*= 0.278).

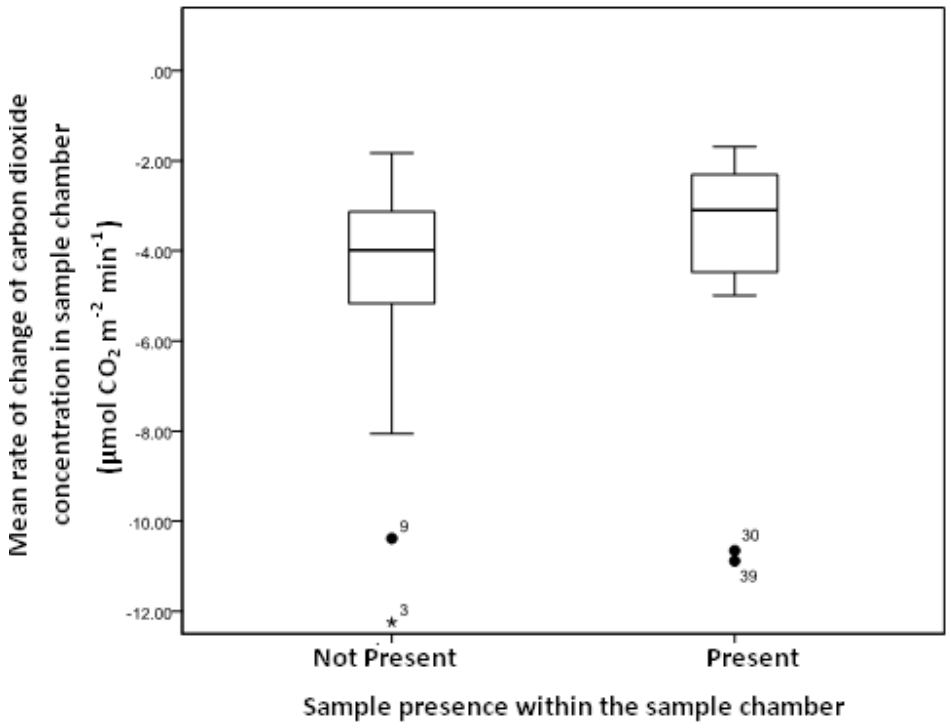

Figure 3.2.2: Boxplot figures illustrating the median rate of change of CO_2 concentration, error bars indicate +/- two standard deviations, over the course of the 10 minute measurement period; while the sample was within the chamber and when it was not. Outlying values are marked and numbered and error bars indicate the total range of values obtained within the 10 minutes (not including significant outliers). Lines within the boxplot indicate the median rate value.

Discussion

The results suggest that there was no difference in the rate of gas-exchange when a sample was present in the sample chamber and when it was not. This may have been due to photoinhibiting levels of light during the preliminary tests or to poor performance of the lichen sample; but as Aubert *et al.* (2007) states, the minimum detection threshold (in a similar IRGA model) is 10 nmol min^{-1} and, retrospectively, many of the results detailed later in this report are levels below this detection value.

More important were the high levels of variation, which would reduce the statistical significance of any results obtained and would require larger sample sizes to readdress the loss of accuracy; which would have taken more time and used more material. The amount of material available was limited as it had to be flown in from Abisko, Sweden. Because of this, it was decided that the IRGA apparatus was not suitable for use in this series of studies.

3.3 The O_2 electrode apparatus

3.3.1 Introduction

In the experiments reported in this dissertation an Oxygraph (Hansatech, King's Lynn, Norfolk, UK) oxygen electrode apparatus was used combined with an S1 Clark type electrode disk and LD2/3 model electrode chamber (Hansatech). The apparatus allows the measurement of changes in the concentration of O_2 within a closed chamber of a known volume. When a lichen sample is placed within the electrode chamber the rate at which O_2 is taken up (respiration) or evolved (photosynthesis) can be inferred from the rate of change of O_2 concentration. This method has been used before to record the response of photosynthesis to changing climatic factors in published studies (Aubert *et al.*, 2007; Badger *et al.*, 1993; Rogers, 1971).

The oxygen electrode methodology has distinct advantages over the use of an IRGA apparatus: firstly the electrode chamber is small and only requires small samples, meaning that the limited store of lichen material available can be conserved. Even though smaller samples are used the electrode apparatus gives the same comparative power as one would receive from larger samples in the IRGA. Secondly as the electrode chamber is a closed system with no air-flow the lichen samples exhibited considerably lower levels of water loss during measurement than in the IRGA chamber (where samples could go from fully hydrated to metabolically inactive within the time required to complete a measurement). The rapid rate of water loss which thalli are subjected to, while being measured, is stated as being a major disadvantage to utilising gas exchange measurement apparatus (Sigfridsson, 1980). As the importance of maintaining a constant hydration state when measuring the affect of temperature and light intensity on the photosynthetic rate of lichen thalli has been highlighted in many studies, and as when using IRGA apparatus this is impossible with small samples, the use of an oxygen electrode apparatus is preferred (Ino, 1985; Lange 2002). Finally, as the chamber is small, there is no need for an air-stirring mechanism, as there is in an IRGA cuvette, the formation of a boundary layer is possible, which may make results closer to those obtained *in situ* (Lange et al., 2001). Importantly the electrode chamber has facilities which allow the internal environment of the chamber to be controlled. An atmosphere with a known concentration of CO_2 can be created by passing pre-mixed gas through the inlet valve. The internal chamber is surrounded by a water bath which can be used to reduce temperature changes within the measuring chamber.

3.3.2 Methods

Simple investigations where performed in order to learn how to use the apparatus. All experimental set-ups were performed following the manufacturer's instructions. In cases where sample thalli were used they were hydrated according to the schedule set out in the methods of section 3.2.2. For the investigation of the effect of light inside the chamber there was no sample within the chamber and the water bath was running at 20˚C. For the effect of closing the chamber of the internal atmosphere data were taken with water bath set at 15˚C and a light intensity of 710 μmol m^{-2} s^{-1}. Temperature was recorded with a thermometer inside the sample chamber.

3.3.3 Results – complications resulting from preliminary investigations

Unfortunately the oxygen electrode apparatus has not, so far, been commonly used within the field of lichen studies. This means that data comparisons which other results to check authenticity are difficult. There were also some difficulties, which were encountered when learning how to use the device, with measuring subjects with such low photosynthetic rates per unit of biomass and there is little precedence in how to fix these problems. There were two complications to which no idela solution was identified: Firstly, orientating the lichen thalli as they would be in their natural setting, within the electrode chamber is impossible with fruiticose lichens due to the small size of the chamber. It is unknown whether this will have had an effect upon the data collected; although any effect is unlikely to be large. And secondly, as with other gas-exchange measurement systems photorespiration may significantly decrease photosynthetic output, and the effect of photorespiration would not be discernable in the results (Sigfridsson, 1980). This can be somewhat avoided by increasing the concentration of CO_2 available for photosynthesis.

A serious problem, which is not mentioned within the literature, is the effect of light on the pressure within the measurement chamber. Turning on the LED system (Hansatech, Ultra-bright LH36 262) increased the temperature within the electrode chamber by up to 0.4˚C if the chamber taps are left closed even with the water bath correcting temperature changes; see Figure 3.3.1. This resulted in a change in pressure within the chamber, inducing the electrode to report a rise in oxygen concentration of 0.2 μmol over the course of 5 minutes (retrospectively this value was equal to the rate of some samples measured). A similar magnitude and time frame of changing chamber air temperature and O_2 concentration was evident after the light source was turned off (Figure 3.3.1). This means that turning the light on and off without balancing the pressure causes a rise and fall (respectively) of 'oxygen concentration' as read by the Oxygraph. In order to counteract this effect the chamber must be allowed to settle after the light is turned on/off for at least 5 minutes before

any measurements can be taken (Figure 3.3.1). This phenomenon is not mentioned in the articles referenced above; Aubert *et al.* (2007); Badger *et al.* (1993) and Rogers (1971), and their assessment of photosynthetic rate and dark respiration rate in lichens may be overestimated.

Additionally, the action of placing a sample inside the sample chamber disrupted the equilibrium required for the apparatus to measure gas-exchange rate. Temperature required approximately 5 min to equilibrate after the chamber was closed; irrespective of the presence of a sample (Figure 3.3.2). With *N. arcticum* thalli inside the chamber, 5 minutes after the chamber was closed the O_2 concentration increased; but without a sample concentration remained constant at this point in time Due to this difference it was concluded that increased O_2 concentration, at 5 min with the sample present was due to photosynthetic O_2 production by the thalli (Figure 3.3.2)

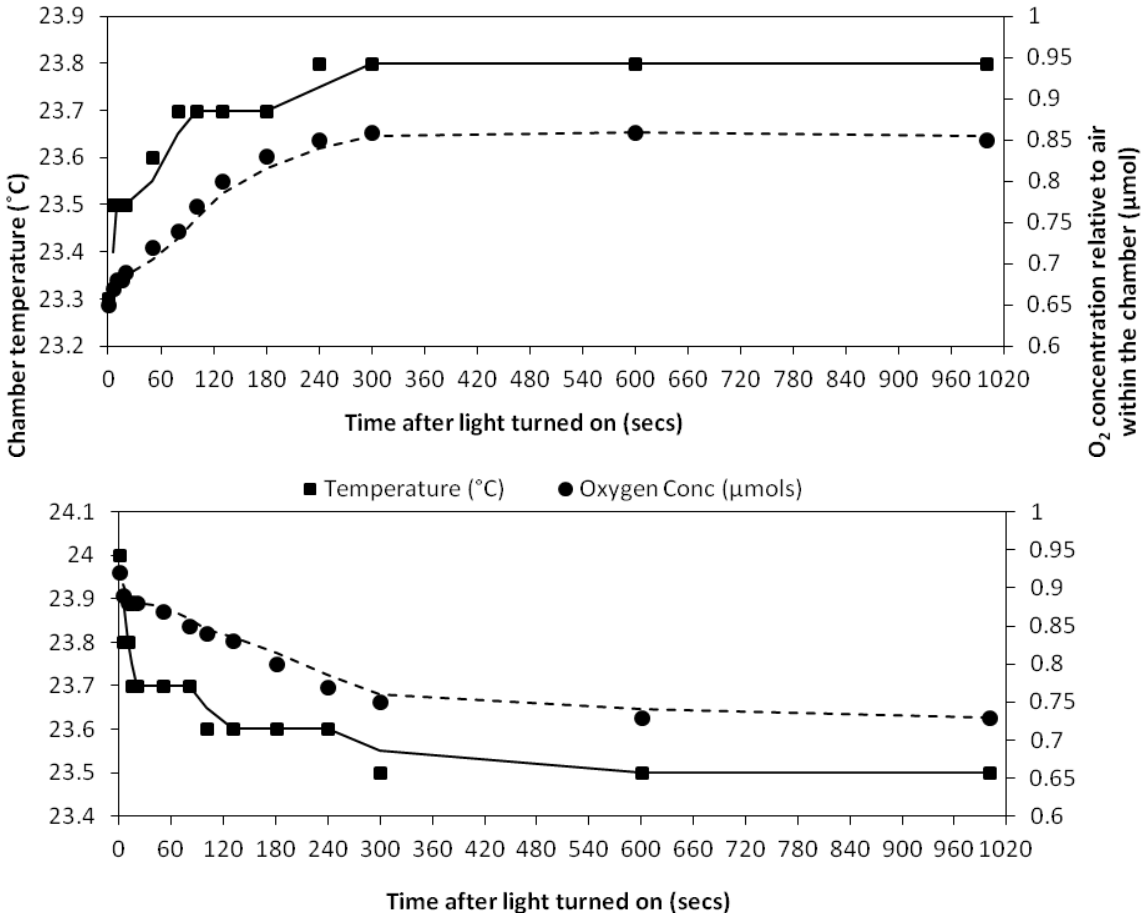

Figure 3.3.1: Graphs plotting the change in chamber O_2 concentration and air temperature following the switching on and off of the light source shining into the sample chamber.

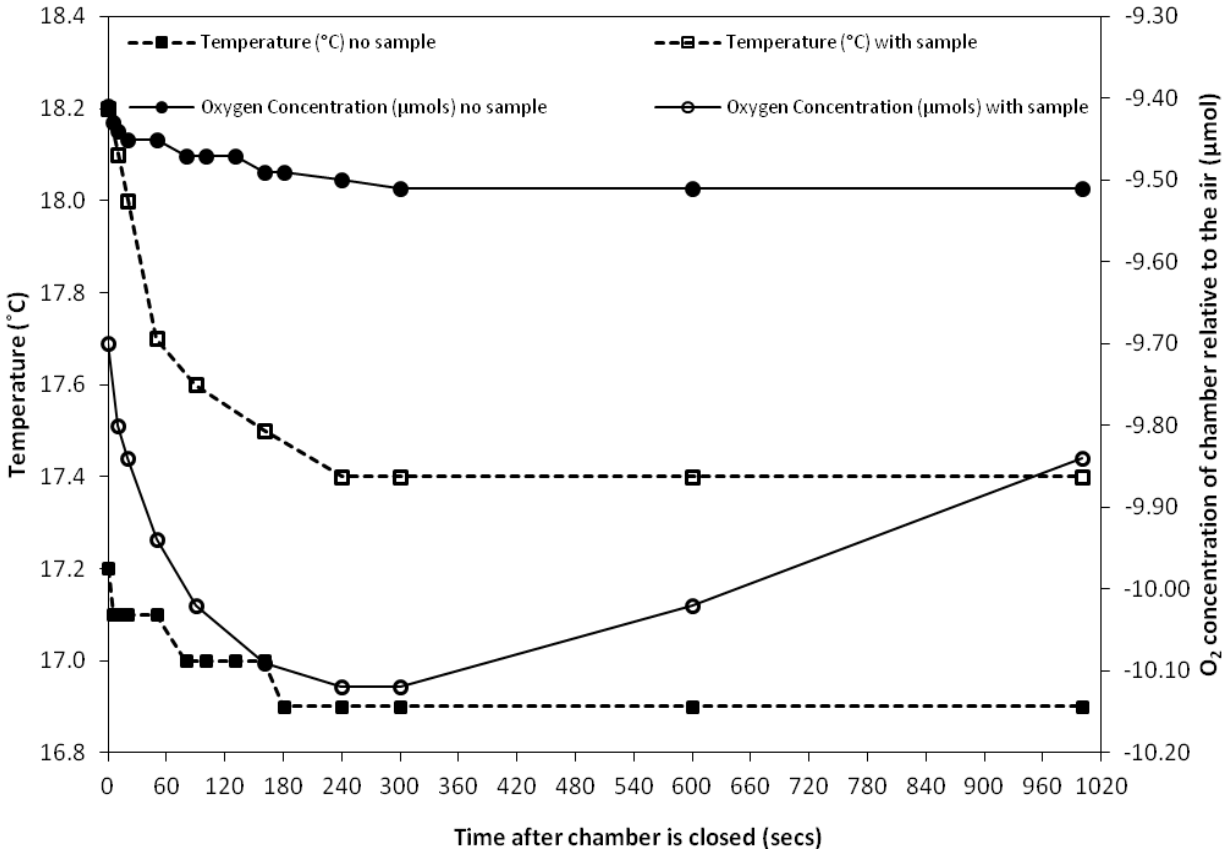

Figure 3.3.2: Graph illustrating the change in mean air temperature and mean O_2 concentration within the sample chamber against time after the chamber was closed. Each point represents a mean of five replicates. Open points indicated data that was recorded while a sample on *N. arcticum* was inside the chamber and filled points while there was not. Error bars have been removed to reduce confusion.

The final main hindrance associated with the use of the Oxygraph apparatus was the prolonged time spent waiting for the apparatus to settle. Therefore data was obtained at a slower rate than would have been obtained with the IRGA apparatus. Three months of daily measurements were required for the collection of the photosynthetic rate data for the studies presented later in this report.

3.3.4 The use of the O_2 electrode apparatus in further chapters

As a result of the lack of previous investigations using the O_2 electrode apparatus the series of preliminary experiments, detailed previously, were required to produce a valid method of measuring the rate of gas exchange of the sample lichens. As a result of these experiments a scientific method was produced that could accurately measure gas exchange with the sample thalli at optimal conditions; producing maximal rates of photosynthesis/respiration at any given set of environmental conditions. This section outlines said method.

The method

The electrode chamber was cooled using a clear glycol/distilled-water mixture (at a 70:30, by volume, ratio) which was run through the clear water jacket using an electric pump. The pump and fluid were incorporated in a cooling apparatus (Geneline cooler, Beckman Coulter, High Wycombe, UK) which was capable of refrigerating the glycol-solution running through the water bath surrounding the measurement chamber to around -10˚C, the minimum air temperature achievable inside the measurement chamber was ~5˚C.

The electrode was set up according to the Hansatech Operations Manual (Hansatech, 2006). 50 % KCl (potassium chloride) solution was used as an electrolyte. The electrolyte was made by mixing four parts deionised water to four parts saturated KCl solution; the saturated KCl solution was formulated by mixing 36 g of anhydrous salt in 100 ml of deionised water at room temperature (Hansatech, 2006). Blue Rizla™ cigarette papers were used as the spacer medium. Once the electrode disk was connected to the Oxygraph system the apparatus was left set-up and the oxygen concentration was recorded by the accompanying computer program (Oxygraph Plus, Hansatech).

A thermometer (T206TC model, Digitron, Torquay, Cornwall, UK) was employed to measure the temperature inside the sample chamber. Calibration was begun when both the temperature readings on the thermometer and the oxygen concentration recorded by the electrode disk were demonstrated to be stable (normally requiring an hour of acclimation to the temperature after initial set-up; as suggested in the operations manual). The air pressure of the room was ascertained using the parametric sensor in the LI-6400 IRGA apparatus. Calibration was automatically processed by the Oxygraph Plus software, requiring only the addition of 1 ml of air into the electrode unit. Due to the change in temperature from the atmosphere of the room to that of the sample chamber the additional air was allowed at least 5 minutes to reach an equilibrium temperature in the chamber. Once the electrode was calibrated the light was set at the intensity required for the following series of measurements. In accordance with the preliminary discovery, regarding the effect light radiation

entering the chamber once the light was turned on, had on the air pressure within the chamber, at least 5 minutes was given before samples were added to the chamber; according to the results illustrated in Figure 3.3.1.

Once the set-up process was complete, a lichen thallus sample was added to the chamber. After addition, a set period of time was required, to allow the thallus and the chamber air to equilibrate with the temperature of the cooling medium, before any rate measurements could be recorded. Preliminary experiments have shown that 5 minutes was sufficient for the mean temperature and the mean O_2 concentration to become constant (Figure 3.3.2). The equilibration period was started after the addition of the CO_2 mixture. The mixture was added at a set rate of 0.5 l min^{-1} for 10 seconds after the placement of each sample. If there was evidence of a pressure change due to changing temperature a gas tap was opened to allow the chamber to equalise and the equilibration timer was restarted.

The change in concentration of O_2 within the chamber was then recorded for a further 4 minutes. Preliminary experiments showed that at the high sensitivity used in this experiment the Oxygraph signal, in many cases, varied as a wave function, hence when two lines of best fit were drawn through two 2 minute sections (with no sample) the mean change in oxygen concentration was zero. The lines of best fit were automatically drawn by the Oxygraph Plus software. For all samples the net change in oxygen concentration during each measurement was taken to be the mean of the two rates resulting from lines of best fit passed through the two 2 minute sections. The chamber air temperature was monitored during each 4minute period using the thermometer. If the value changed by more than 0.2˚C then the measurement was restarted from the beginning, this was a precaution taken due to the results shown in Figure 3.3.1.

3.3.5 Hydration

Introduction

It has been shown in the previous chapters that the metabolic rate of lichens is significantly affected by its previous hydrological environment. Therefore, a preliminary experiment was performed to ascertain the hydration procedure that maximised the lichen's metabolic rate.

Methods

In order to attain a fully hydrated state, sample thalli (0.5 g) of each species were submerged in distilled water (distilled water was used in case changing mineral contents of rain water or tap water affected subsequent NP or DR) for 5 minutes, after having been in a state of desiccation. 5 minutes has previously been shown to be enough time for the thalli of the lichen *L. muralis* to absorb ~90% of its maximum water content (Seaward, 1976). After submersion, samples were placed between two pieces of wet tissue paper to stop desiccation during storage. This method of storage is used in Bartak *et al.* (2008) and Gasulla *et al.* (2009) to allow lichen thalli to be maintained in a maximally hydrated state whilst being stored. Tissue paper, which had undergone very little processing in its production, was used to maintain lichen wetness to minimise complications arising from seepage of chemicals, from the paper, that may affect the metabolism of the lichens studied.

Measurements were taken from five samples for *C. stellaris*, and six of each of the other species. During storage thalli were kept at 15˚C in low light conditions (circa 50 µmol m^{-2} s^{-1} light intensity). *C. stellaris* samples were measured after 0, 24 and 48 hours of storage in a fully hydrated state. *N. arcticum* and *C. mitis* samples were measured after every five hours in storage, up to 30 hours total.

Samples were measured according to the manufacturer's instructions. The internal environment of the sample chamber was maintained at an air temperature of 10˚C and a light intensity of 510 µmol m^{-2} s^{-1}. After each measurement the samples were rehydrated and stored as described above.

Results

One day wet storage prior to placement within the chamber provided maximal rates of NP in *C. stellaris* (Figure 3.3.3) and *N. arcticum* samples; and near maximal rates for *C. mitis* thalli (Figure 3.3.4). Storage past 24 hours increased the standard error of photosynthetic rate data.

Discussion

Keeping *C. stellaris* hydrated for 24 hours prior to measurement has been found to provide optimal rates of NP; suggesting the results are valid (Groulx and Lechowicz, 1987). Due to the results of this preliminary experiment, all thalli used in the later experiments were subjected to the hydration schedule provided above and allowed 24 hr storage before metabolic rate measurements were taken.

After the final measurement, samples were allowed to desiccate as long-term storage in the hydrated state has been shown damage thalli (*Chapter 2*). This should reduce the problems associated with reusing sample thalli.

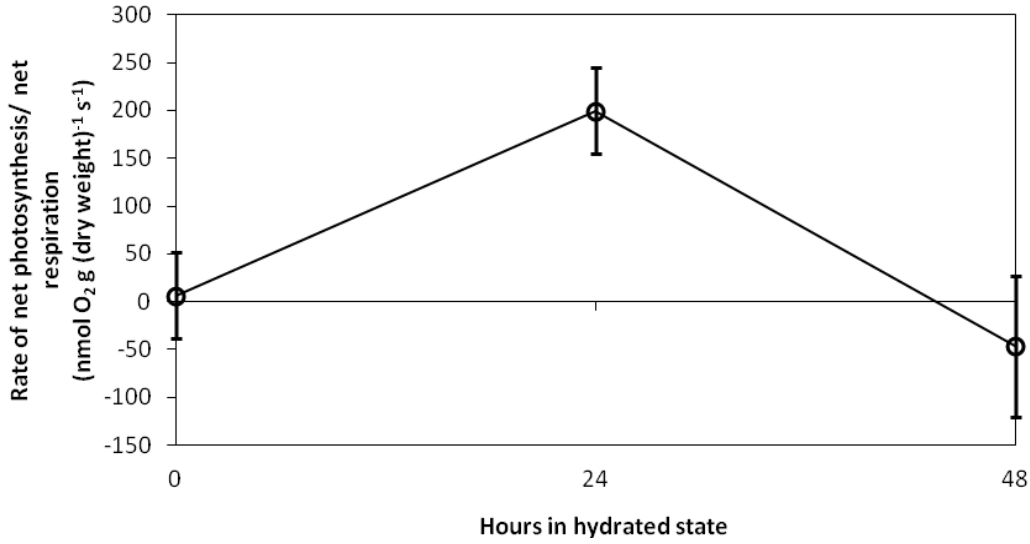

Figure 3.3.3: The mean rate of photosynthesis of *C. stellaris* thalli at increasing lengths of storage in a fully hydrated state. Error bars indicate +/- one standard error.

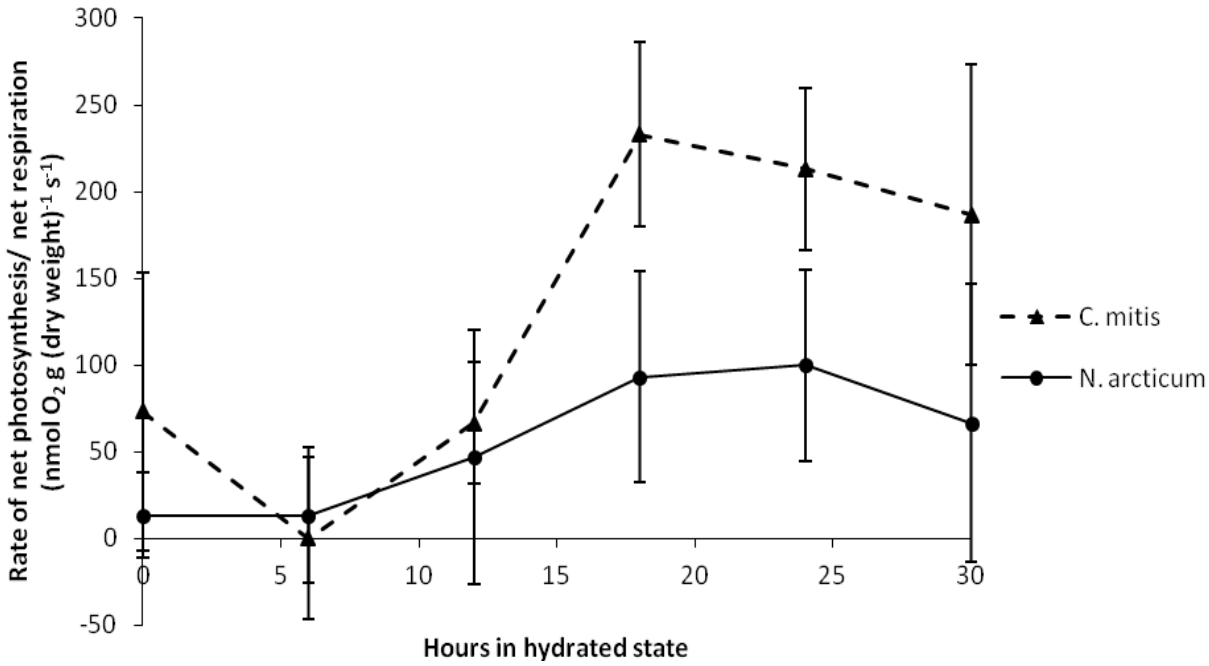

Figure 3.3.4: Mean photosynthetic rate of sample thalli of two species of lichens at increasing lengths of time stored in a fully hydrated state. Error bars indicate +/- one standard error.

Chapter 4

The effect of short-term exposure, to varied air temperature, on the light response of photosynthesis and dark respiration, of four species of lichens: *N. arcticum*, *C. mitis*, *C. stellaris* and *C. rangiferina*

4.1 Introduction

Chapter 1 has shown that both temperature and light intensity significantly affect the rate of NP of lichens. Metabolic rate data, from studies of the effect of environmental conditions on NP and DR in lichens, have, however, been primarily collected using IRGA apparatuses. IRGA apparatuses do not adequately conserve the poikilohydric water status of lichen thalli and dry out very quickly in IRGA sample chambers. It has been highlighted in many studies that in order to properly study the effect of other climatic factors on metabolism of lichens, the water content of sample thalli, which has been shown to significantly affect the rate of metabolism (*Chapter 1*), must be kept constant (Adams, 1971; Coxson *et al.*, 1983). In this regard the oxygen electrode apparatus is superior to their IRGA, as water content of thalli is lost at a much slower rate inside the electrode chamber than in the IRGA sample chamber. It is hypothesised that this study will give a better idea of how temperature affects arctic-heath lichen species than experiments conducted using IRGA apparatuses.

It has been found in a species of lichen (*L. muralis*) and is a common phenomenon in higher plants (Tenhunen *et al.*, 1984), that the temperature not only affects the rate of NP and DR (*Chapter 1*) of a thallus but also affects the level of photoinhibition that occurs within the thallus photosystems and the efficiency of photosynthesis (Lange, 2002). This is a relatively unstudied phenomenon in arctic lichens, yet it could be an important mechanism by which climate affects the rate of lichen metabolism, and subsequently could affect the survival of lichen species. In this report the presence of a significant interaction between the effect of temperature and light intensity has been analysed in order to establish the presence of an effect of temperature of the effect of light intensity of thallus NP.

This chapter is a report based on a series of measurements of the mean rate, of both net photosynthesis and dark respiration, at a series of temperatures and light intensities, for the four species outlined in the title. The report details: the method by which an Oxygraph oxygen electrode apparatus was used to obtain the rate measurements, the data acquired through the use of the apparatus, the results of statistical analyses performed to determine the significance of the factors which determined metabolic rates and a brief discussion of the importance of the results.

4.2 Method

The Oxygen electrode apparatus was used to measure the rate of NP and DR of 10 sample thalli, for each of the four lichen species, at a range of air temperatures and light intensities. The finalised method by which NP and DR were measured is described in the previous chapter. Prior to measurement, sample thalli were keep fully hydrated for 24 hours; as per Chapter 2. Thalli were blotted of extraeneous water and weighed gravimetrically with an electric balance before being placed within the electrode chamber.

CO_2 concentration within the electrode chamber was regulated at 1500 ppm by injecting gas at this concentration directly into the chamber. The hydration method described in Chapter 2 did not provide a constant wet weight of thalli before measurement (see Section 3.3.). Hydration state is known to affect the rate of NP and DR due to supersaturation depression (*see introduction*), but higher CO_2 availability reduces the effect of supersaturation depression on NP (Coxson *et al.*, 1983). To minimise the effect of differing wet weights between measurements, the analysis was done at a high enough CO_2 concentration where response curve of NP to hydration state has been shown to be flat in other species of lichens (Lange, 2002). As higher CO_2 concentrations produce flatter photosynthesis-hydration response curves, metabolic rates obtained by gas exchange apparatus under high concentration conditions are of a similar magnitude to results obtained at optimum hydration in *in situ* conditions (380ppm) (Coxson *et al.,* 1983). This means that the results obtained by this method can be considered to be optimal rates for each species at any given air temperature/light intensity combination.

For each species the rates of NP and DR were measured at three different air temperatures (5˚, 10˚ and 17˚C) and five light intensities (0, 170, 340, 510 & 760 µmol m^{-2} s^{-1}). The three temperatures represent a spread of temperatures which the species would be exposed to *in situ* over the course of the year in the ecosystem which the samples were collected from (Baxter *et al.*, unpublished). Lower temperatures were attempted but resulted in the cooling fluid freezing.

Dark respiration rates were obtained at 0 µmol m^{-2} s^{-1}. 0-760 µmol m^{-2} s^{-1} provided a wide range of light intensities that thalli of these species will encounter *in situ* and although light intensities in Swedish arctic-alpine habitats can reach higher levels (up to 1000 µmol m^{-2} s^{-1}), during even the sunniest months of the year (June through September) intensities of higher than 700 µmol m^{-2} s^{-1} occur less than 10% of the time (Sonesson *et al.*, 1992).

One set of 10 sample thalli were measured per day. On each day photosynthetic rates of the thalli of a single species were recorded at a single air temperature and light intensity. In order to stop

the conditions of the previous experiment affecting the data obtained on any given day, sample thalli were left in storage for a period of no less than four days before being measured again. A randomised table of light intensity and air temperature combinations was created and the samples were measured in the order generated by the table to reduce the possibility of external factors affecting the results.

Statistical analyses:

The presence of a significant change in the wet weight, prior to placement within the electrode chamber, over the course of the experimental period, has been tested for using linear regression analysis (SPSS).

Metabolic rate data obtained from this experiment, for the purpose of the two-way ANOVA analysis, have been collated into data sets according the environmental conditions under which values were collected. All values of sample-chamber temperature at time of measurement are accurate to ± 0.5˚C. Light intensity groupings are based on level of light which reaches the lichen thalli's surface and unfortunately not the level of light which reaches the photobionts photosystems. For the purpose of the test, dark respiration values have been omitted, resulting in 12 groups for comparison: four groups of light intensities at each of the three air temperatures. The dependant variable in the analysis is rate of change of O_2 concentration within the chamber (in nmol min^{-1} g (dry weight)$^{-1}$) and the independent variables are light intensity (in µmol m^{-2} s^{-1}) and air temperature (in ˚C). Species photosynthetic responses, to the independent variables, have been compared individually by two-way ANOVA (SPSS Statistics 17.0, SPSS Inc, Chicago, Il, USA).

The significance of the effect of temperature on the light compensation point of photosynthesis as been analysed using an ANOVA test (SPSS). Compensation points have been taken as the x intercept of each individual thalli from a line of best fit drawn between NP at 0 µmol m^{-2} s^{-1}, 170 µmol m^{-2} s^{-1} and 340 µmol m^{-2} s^{-1}.

4.3 Results

4.3.1 Change in wet weight over the course of the experimental period

For each species a linear regression analysis was performed to test whether wet weight of the sample thalli changed significantly over the experiment period. This is important, as if there has been a significant directional change in the mean wet weight of the sample thalli of a species, there may have been a loss of biomass during the experiments. It is important that the changes in photosynthetic rate of the thalli, ascribed below to changing air temperature and light intensity, are not artefacts of a loss of biomass, which would cause rate data to have been underestimated.

In Figures 4.3.1 (a, b & c) it can be seen that neither *C. mitis* nor *C. stellaris* nor *N. arcitcum* exhibited a significant permanent reduction of sample thallus wet weight over the course of the experimental process; linear regression analysis found no significant directional change over time. There is no evidence, in these three species, that any single sample exhibited a significant gain or loss of biomass as there were no outlying values for wet weight of sample thalli; i.e. at each date of variation in wet weight of a magnitude that could have been caused by a difference of the amount of water taken up by a sample. It can be assumed that for *C. mitis*, *C. stellaris*, and *N. arcitcum*, the biomass of each sample did not vary of the course of the experiment and as such the final dry weight obtained for each thallus was valid for use at all measurements.

However in Figure 4.3.1 (d) a large reduction in the wet weight of the thalli, of *C. rangiferina* samples, over the course of the experiment is described by the line of best fit. The linear regression analysis results suggest that mean wet weight of the *C. stellaris* thalli changed significantly over time. In Figure 4.3.1 (d) the actual thalli which have lost biomass over the course of the experiment can be identified by the dotted trend lines. As samples 1, 5 and 7 exhibited a large negative trend in wet weight over time it can be stated that the dry weight of these samples has changed. As dry weight cannot be established retroactively these samples have been removed from the metabolic rate results in this Chapter. The dark respiration data was acquired on the same day as the dry weight of sample thalli and analysis of this data alone includes values for 1, 5 and 7.

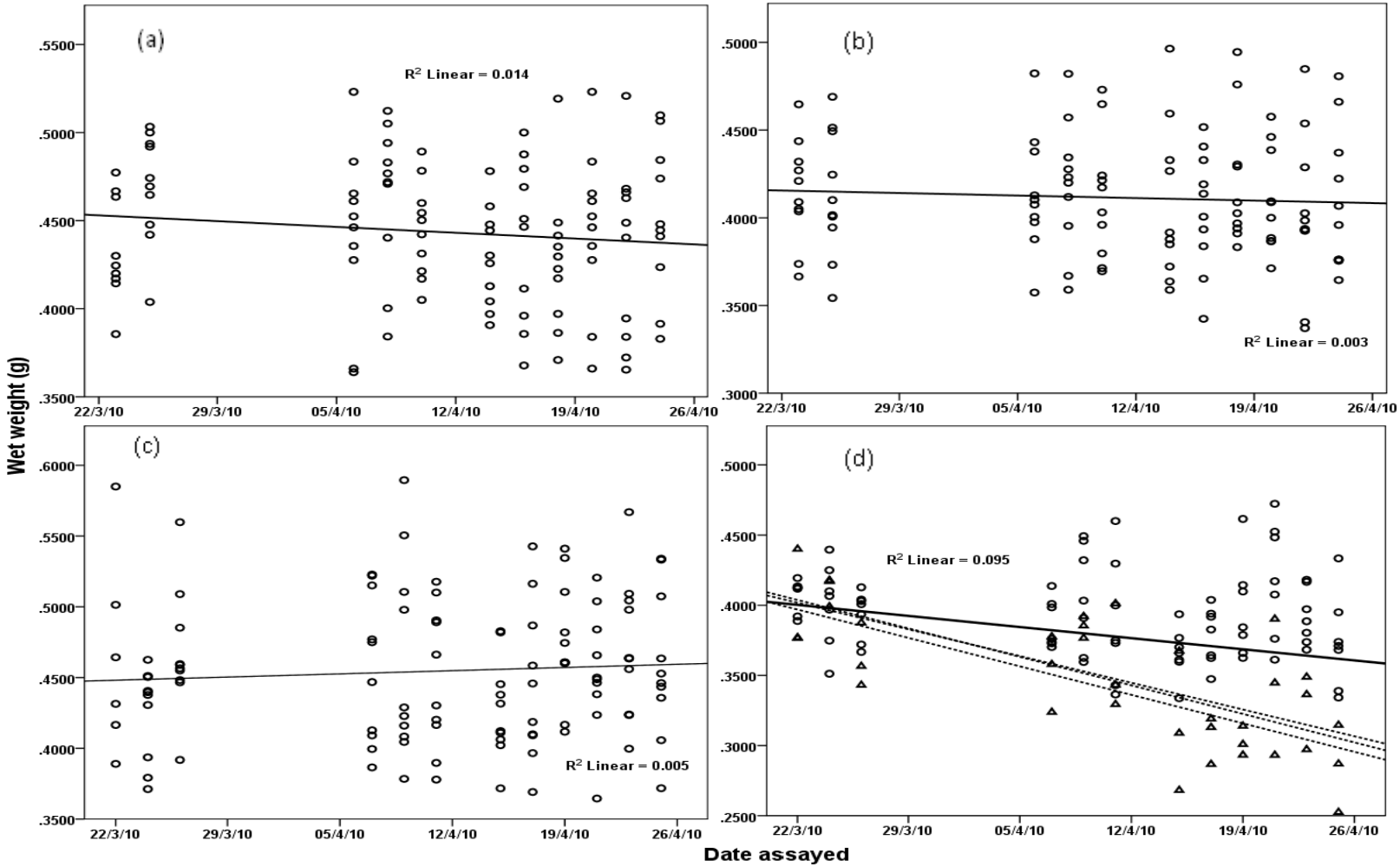

Figure 4.3.1: Graphs illustrating the wet weight of each sample thallus, prior to measurement within the electrode chamber, on the date of assay. Each graph gives the wet weights of a different species: (a) *N. arcticum*, linear regression analysis ($F_{(1\ 107)}$= 1.469, *P*= 0.228); (b) *C. mitis*, ($F_{(1\ 107)}$= 0.367, *P*= 0.546) (c) *C. stellaris*, ($F_{(1\ 107)}$= 0.563, *P*= 0.455); (d) *C. rangiferina*, ($F_{(1\ 107)}$= 12.394, *P*= 0.001). In graph (d) dotted trend lines indicate the directional variation, and open triangles the actual values, of the three samples that exhibited a noticeable reduction in wet weight over the experiment period. Samples which exhibited to noticeable trend are represented with open circles.

4.3.2 The response of photosynthesis/ respiration to the variation of external climatic variables

The photosynthetic response to light intensity curves, at each of the three air temperatures, for *N. arcticum, C. mitis, C.stellaris* and *C. rangiferina*, are presented along with the results of the two-way ANOVA results. The mean light compensation values for each species at each temperature and the results of an ANOVA testing the effect of air temperature on the light compensation point of photosynthesis have also been provided. Kolmogorov-Smirnov tests have found that rate of net photosynthesis data are normal for all species (Table 4.3.2). However the Levene's test revealed that the recorded rates of net photosynthesis, recorded for *C.* mitis and *C. stellaris*, displayed a significant level of heterogeneity of error variance (Table 4.3.2). Although this violates a basic assumption of AVONA analysis, in cases where Equation 2 is satisfied then ANOVA analysis can be undertaken under normal parameters (Pallant, 2007). In the cases for all experiments conducted on these species N= 10 every group; which satisfies the above equation, therefore effects can be considered significant if *P* is less than 0.05 (i.e. $\alpha = 0.05$).

Equation 2: $$\frac{N\ of\ smallest\ group}{N\ of\ largest\ group} < 1.5$$

Nephroma arcticum:

Both air temperature and light intensity significantly affected the rate of photosynthesis in *N. arcticum* thalli (Table 4.3.2). The analysis also suggests that there was a significant interaction effect between the two factors (Table 4.3.2), which is evident in Figure 4.3.2 (a) by the change in the shape of the light response curve at each temperature. Post-hoc Tukey test results suggest that the mean photosynthetic rate at 17˚C was significantly lower than the other two temperature groups and that rates obtained at 10˚C ($P< 0.001$) and 5˚C ($P< 0.001$) were not significantly different from one another. Also the mean rate of photosynthesis of thalli at 170 µmol m^{-2} s^{-1} varied significantly from the rate at 340 µmol m^{-2} s^{-1} ($P= 0.001$) and 510 µmol m^{-2} s^{-1} ($P= 0.023$), however no other light intensity group comparisons were significantly different. The species exhibited a maximal rate of NP at 5˚C and ~340µmol$m^{-2}s^{-1}$ however at the higher air temperatures the light saturation point (the value at which light is no longer the limiting factor of photosynthetic rate) increases to ~510µmol$m^{-2}s^{-1}$ (Table 4.3.1).The light compensation point (the light intensity at which respiratory oxygen uptake

and photosynthetic oxygen evolution are equal) also significantly increased with rising air temperature (Table 4.3.3) (Tenhunen *et al.*, 1984).

Cladina mitis:

The two-way ANOVA test suggests that both air temperature and light intensity had a significant direct effect on the rate of photosynthesis exhibited by *C. mitis* thalli, in this species there was no evidence of a significant interaction effect (Table 4.3.2). The lack of interaction effect, between light intensity and air temperature is visible in Figure 4.3.2 (b), where at all temperatures thalli exhibited similarly curved responses. At all air temperatures optimum light intensity was 340 µmol m^{-2} s^{-1}. A post-hoc Tukey test results suggest that the mean rates obtained at 17˚C are significantly lower than those obtained at 5˚C (*P*= 0.015) and almost significantly from those obtained at 10˚C (*P*= 0.062) (the power of the test to differentiate between data obtained at each air temperature may dramatically increased with more repeats). Furthermore, data recorded at the optimum light intensity (340µmol$m^{-2}$$s^{-1}$) seem to significantly differ from data obtained at the other lights conditions; even though there is not much difference in mean rates at 17˚C. The light compensation point significantly increased with increasing air temperature (Table 4.3.3).

Cladina stellaris:

The two-way ANOVA results suggest that both air temperature and light intensity significantly affected the rate of oxygen evolution of thalli of this species; there was also a significant interaction between the two factors (Table 4.3.2). The interaction effect is not as clear in the graph for this species as it is in the other species, however Figure 4.3.2 (c) illustrates that at 17˚C, photosynthesis was reduced and did not respond to any change light intensity (apart from no light conditions). The suppression of photosynthesis at 17˚C was significant in the post-hoc Tukey test results (compared to 10˚C where *P*= 0.004 and 5˚C where P= 0.007). Unfortunately, due to the nature of the experiment, it is impossible to conclude whether, at 5˚ and 10˚C, the optimum rate of photosynthesis actually peaked between 340 µmol m^{-2} s^{-1} and 510 µmol m^{-2} s^{-1}, or there was indeed a plateau between the two; as suggested by Figure 4.3.2 (c). It is worth noting that with this species that the 5˚ & 10˚C light response curves provided almost indistinguishable O_2 evolution rates, very similar rates of dark respiration and close light compensation points; although light compensation points were significantly higher at warmer temperatures (Table 4.3.3).

Cladina rangiferina:

The results of the two-way ANOVA suggest that the rate of photosynthesis, by *C. rangiferina* thalli, was significantly affected by both air temperature and light intensity (Table 4.3.2). The response of net photosynthesis rates to variable light intensity was significantly different at different air temperatures (Table 4.3.2), the significant interaction effect further is evident in the Figure 4.3.2 (d), where the shape of the light intensity response of photosynthesis curve changes at 10˚C. Although the 5˚ and 17˚ exhibit a similar shape to their response curves a Tukey test finds the mean rates to have differed significantly between the two temperatures ($P < 0.001$), furthermore the 5˚ and 10˚C groups are not suggested to have had significantly dissimilar mean rates ($P = 0.665$). The maximal rate of photosynthesis in this species occurs at 10˚C and 340µmol$m^{-2}s^{-1}$ although at lower light intensities (i.e. 170µmol$m^{-2}s^{-1}$) the temperature optimum appears to be at 5˚C. At 17˚C photosynthesis appears to be depressed at all light intensities. Warmer temperatures resulted in higher light compensation of photosynthesis points (Table 4.3.3).

Species comparison of important photosynthetic characteristics:

From analysing Table 4.3.1 it can be seen that: all species achieved maximum photosynthetic rate at 5˚C, apart from *C. rangiferina* which achieved highest rate of NP at 10˚C. For *C. rangiferina* and *N. arcticum* temperature affected the optimal light intensity of photosynthesis; but, in *C. mitis* and *C. stellaris* optimal light intensity was 340 µmol m^{-2} s^{-1} regardless of temperature. In all four species analysed in this experiment maximal NP achieved was lowest at 17˚C.

Table 4.3.1: The optimum light intensity of mean net photosynthesis and the peak mean photosynthetic rate achieved, for all species, at each temperature.

Species	Air temperature (˚C)	Optimum light intensity (µmol m^{-2} s^{-1})	Peak photosynthetic rate (nmol O_2 min^{-1} g (dry weight)$^{-1}$)
N. arcticum	5	340	227
	10	510	133
	17	510	127
C. mitis	5	340	240
	10	340	200
	17	340	27
C. stellaris	5	340	287
	10	340	273
	17	340	100
C. rangiferina	5	510	233
	10	340	373
	17	680	100

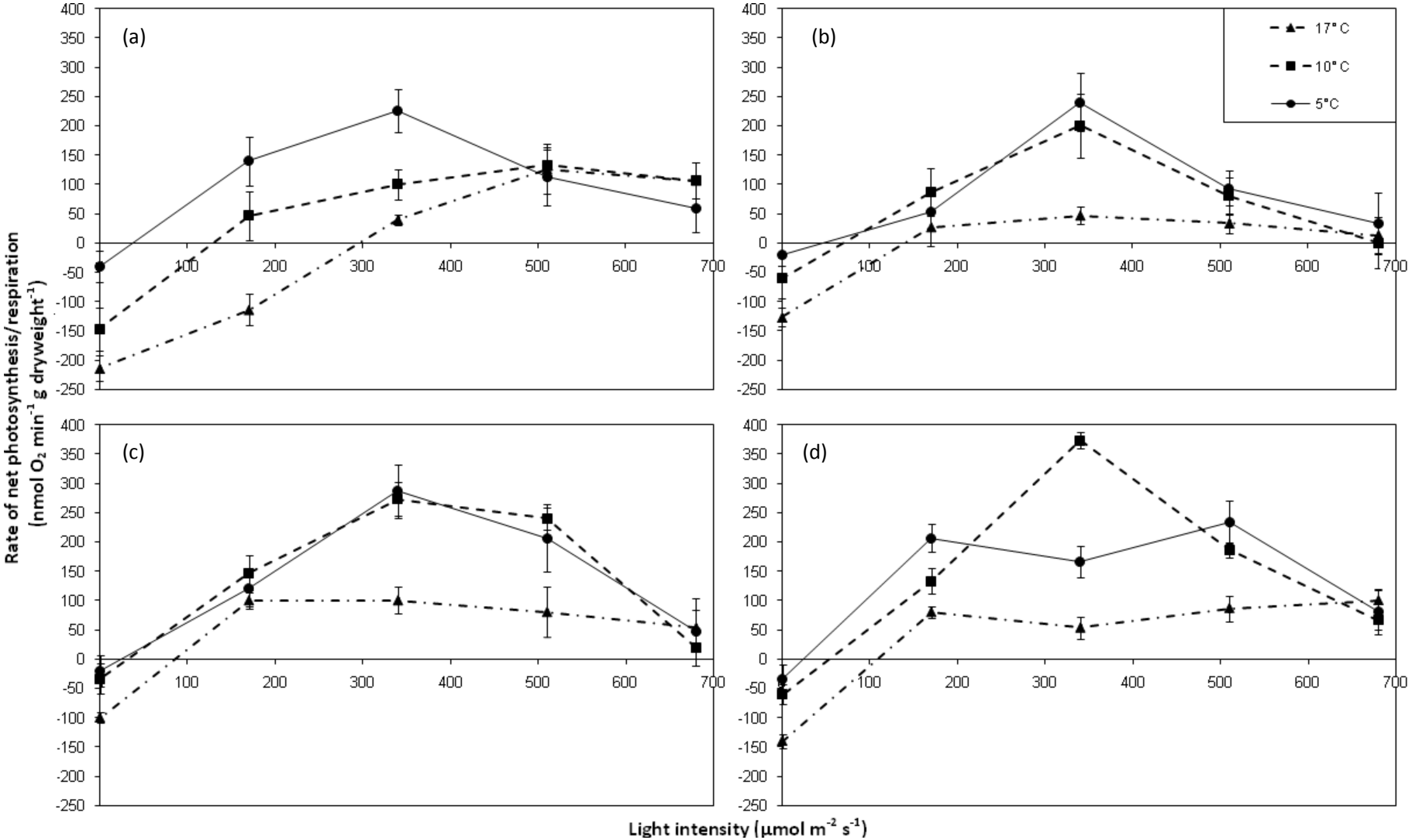

Figure 4.3.2: Graph depicting mean rate of change of O_2 concentration in the oxygen electrode chamber, while sample thalli were present, against light intensity reaching thallus cortex. Values are given at all three temperatures. Positive values indicate that oxygen is being produced by the thallus and that net photosynthesis was occurring; negative values indicate net respiration. Data describe mean rates of net photosynthesis/ respiration of 10 sample thalli, error bars indicate +/- one standard error. (a) *N.arcticum*, (b) *C. mitis*, (c) *C. stellaris*, (d) *C. rangiferina*.

Table 4.3.2: Results of two-way ANOVA testing for the significance of the effect of air temperature and light intensity on mean photosynthetic rate of all four lichen species, and for the presence of a significant interaction effect between the two independent factors. Significant results are marked with an *. Tests of data normality and error homogeneity are also displayed.

	Kolmogorov-Smirnov test			Levene's homogeneity of variance test			Two-way ANOVA			
Species	N	Z value	*P* value	df	F value	*P* value	Independent factor	df	F value	*P* value
N.arcticum	120	0.624	0.830	11, 108	1.021	0.433	Air temperature	2, 108	9.326	< 0.001*
							Light intensity	3, 108	5.358	0.002*
							Interaction effect	6, 108	4.659	< 0.001*
C. mitis	120	1.132	0.154	11, 108	2.362	0.012*	Air temperature	2, 108	4.510	0.013*
							Light intensity	3, 108	8.262	< 0.001*
							Interaction effect	6, 108	1.423	0.213
C. stellaris	120	0.909	0.380	11, 108	2.459	0.009*	Air temperature	2, 108	6.783	0.002*
							Light intensity	3, 108	13.045	< 0.001*
							Interaction effect	6, 108	2.261	0.043*
C. rangiferina	84	0.737	0.648	11, 72	1.568	0.127	Air temperature	2, 72	16.740	< 0.001*
							Light intensity	3, 72	8.990	< 0.001*
							Interaction effect	6, 72	9.523	< 0.001*

Table 4.3.3: The mean light compensation point of photosynthesis, for all four species studied, at all temperatures and the results of an ANOVA testing for the significance of the effect of temperature upon the light compensation point of photosynthesis. Compensation point values are given as means of the x intercept values of individual thalli, for each species at each temperature.

				Kolmogorov-Smirnov test			Levene's homogeneity of variance test		One-way ANOVA	
Species	Air temperature (˚C)	Mean light compensation point (μmol m^{-2} s^{-1})	Standard error (μmol m^{-2} s^{-1})	N	Z value	*P* value	df	F value	*P* value	F value	*P* value
N.arcticum	5 10 17	60.2 117.8 293.3	27.5 19.9 19.1	30	0.720	0.677	2, 27	0.523	0.598	58.233	< 0.001*
C. mitis	5 10 17	51.3 86.0 164.0	17.6 25.4 13.9	30	0.618	0.840	2, 27	1.970	0.159	16.849	< 0.001*
C. stellaris	5 10 17	37.5 49.6 86.8	19.0 25.1 12.6	30	0.858	0.454	2, 27	2.365	0.113	3.453	0.046*
C .rangiferina	5 10 17	19.1 64.6 100.8	13.1 17.7 8.8	21	0.865	0.443	2, 18	5.059	0.018*	9.278	0.002*

Dark respiration

Dark respiration significantly increased a greater temperatures; ANOVA results testing for statistical significance of the effect temperature had on dark respiration: *N.arcticum* ($F_{(2\ 27)}$= 8.304, *P*=0.002), *C. mitis* ($F_{(2\ 27)}$= 4.627, *P*=0.019), *C. stellaris* ($F_{(2\ 27)}$= 3.539, *P*=0.043), *C. rangiferina*: ($F_{(2\ 27)}$= 10.899, *P*<0.001). Error bars indicate +/- one standard error (N= 7-10). The Cladina spp. exhibited reduced respiration rates compare to *N. arcticum*, as well as an exponentially increasing relationship with temperature; while *N. arcticum* exhibited a saturating response.

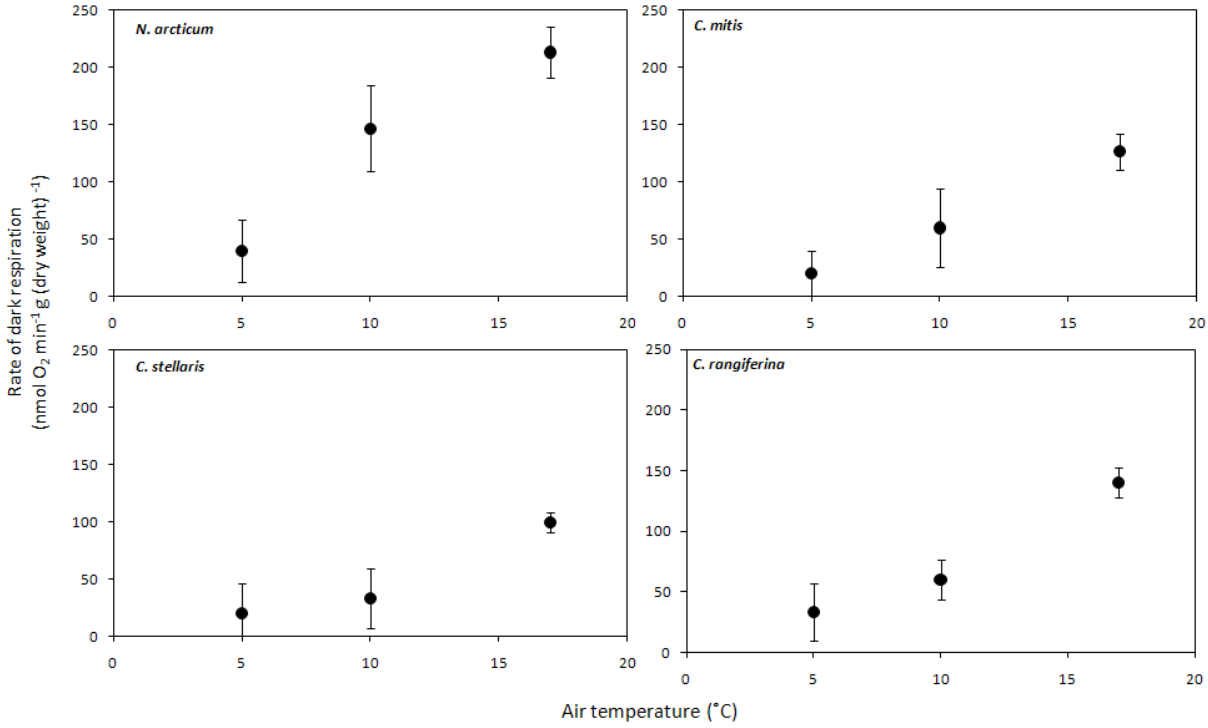

Figure 4.3.3: A series of scatter plots with mean rate of dark respiration for each species (taken as the rate of change in oxygen concentration, while there was no light, within the chamber) at three air temperatures.

Gross Photosynthesis

Removing the effect of temperature on respiration provides gross photosynthetic rate (Figure 4.3.4); resulting in a much clearer relationship between temperature and photosynthetic rate. For all species GP at 680 µmol m^{-2} s^{-1} light intensity was highest at 17 ^{o}C.

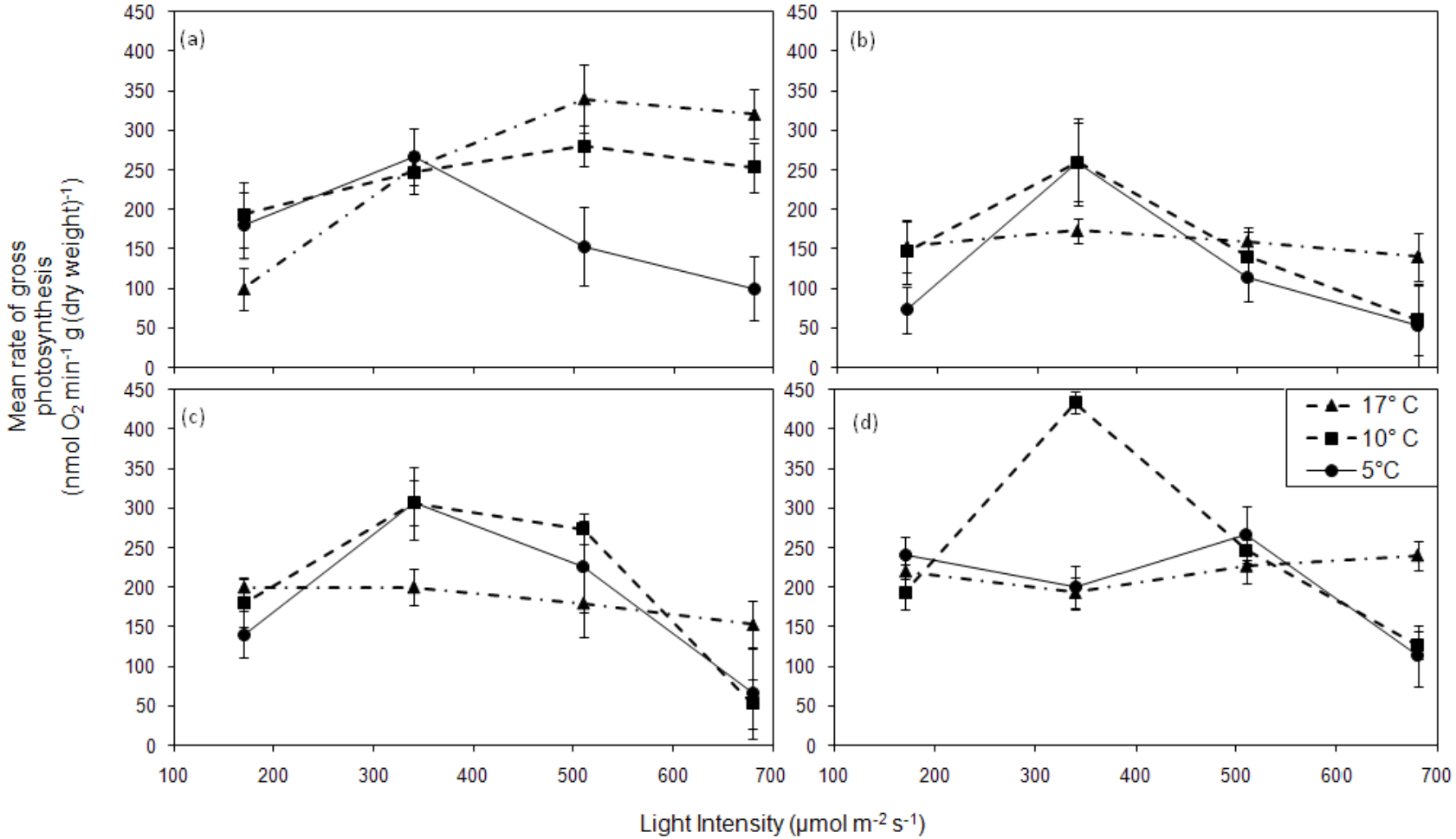

Figure 4.3.4: Plots depicting the mean rates of gross photosynthesis (net photosynthesis – dark respiration) of all four species at different temperatures and light intensities. Error bars indicate +/- one standard error of the mean rate of net photosynthesis (N=7-10). (a) *N.arcticum*, (b) *C. mitis*, (c) *C. stellaris*, (d) *C. rangiferina.*

4.4 Discussion

4.4.1 Accuracy of the oxygen electrode apparatus

The results obtained from the oxygen electrode apparatus appear to corroborate similar experiments performed on the effect of temperature and light on photosynthesis and dark respiration in lichens. Therefore we can be confident that the results obtained in this experiment reflect actual biological processes and are not artefacts of the method. This conclusion is supported to two patterns evident within the results:

Firstly dark respiration rates of lichen thalli, in all species, increase with environmental warming (see Figure 4.3.3). An ANOVA analysis found that the effect air temperature had on thalli DR was significant for all species, see Figure 4.3.3, which implies a direct causation (Sharp, 1983). The difference between the rates of dark respiration at the various temperatures is similar values obtained in experiments, investigating the relationship between temperature and rate of photosynthesis, which also used the oxygen electrode apparatus (Aubert *et al*., 2007). The relationship seems to be constant between species with each exhibiting a roughly exponential increase in the rate of DR with each rise in air temperature (Figure 4.3.3). The rates of DR, for the four species, are directly comparative to data obtained by Aubert *et al.* (2007), in which an oxygen electrode apparatus was used and so the units of NP and DR are the same (Figure 4.4.1). The rate of NP and DR of *X. elegans* thalli, an alpine species which exists in similar environmental conditions, are illustrated in Figure 4.3.2, it is evident that *C. stellaris* also exhibited peak net photosynthetic rate at 10˚C under a ~500 µmol m^{-2} s^{-1} light intensity scenario. The temperature compensation points for both species are under 20˚C. It is quite obvious that NP rate of *C. stellaris* in Figure 4.4.1, which had a consistently higher mean rate than other species studied, was lower than that peak rates found by Aubert *et al.* (2007). Unfortunately there are, to my knowledge, no papers with which this data can be quantifiably compared to ascertain due to the lack of recent studies using oxygen electrode apparatus, and whether the depressed rate of NP is a result of species differences or a result of the methodology.

Secondly all samples exhibit light response of photosynthesis curves as would be expected for Arctic-tundra lichens. All species exhibit a response comparable (both in shape of the curve and the magnitude of the rate of DR and NP) with studies performed on other cold-adapted species. An in depth comparison of the results obtained in this studies and other studies performed on similar species can be found later in this section.

Due to the similarities evident in the results obtained above with the results of similar studies using different IRGA apparatus, it can be concluded that the use of the oxygen electrode apparatus is valid method of measuring NP and DR in lichens. If one considers the advantages of the oxygen electrode apparatus described in Chapter 3 and argued in Aubert *et al*. (2007) then perhaps oxygen electrode use could be considered to be more appropriate for use in a laboratory setting than IRGAs; where maintaining a consistent environment for analysis is difficult.

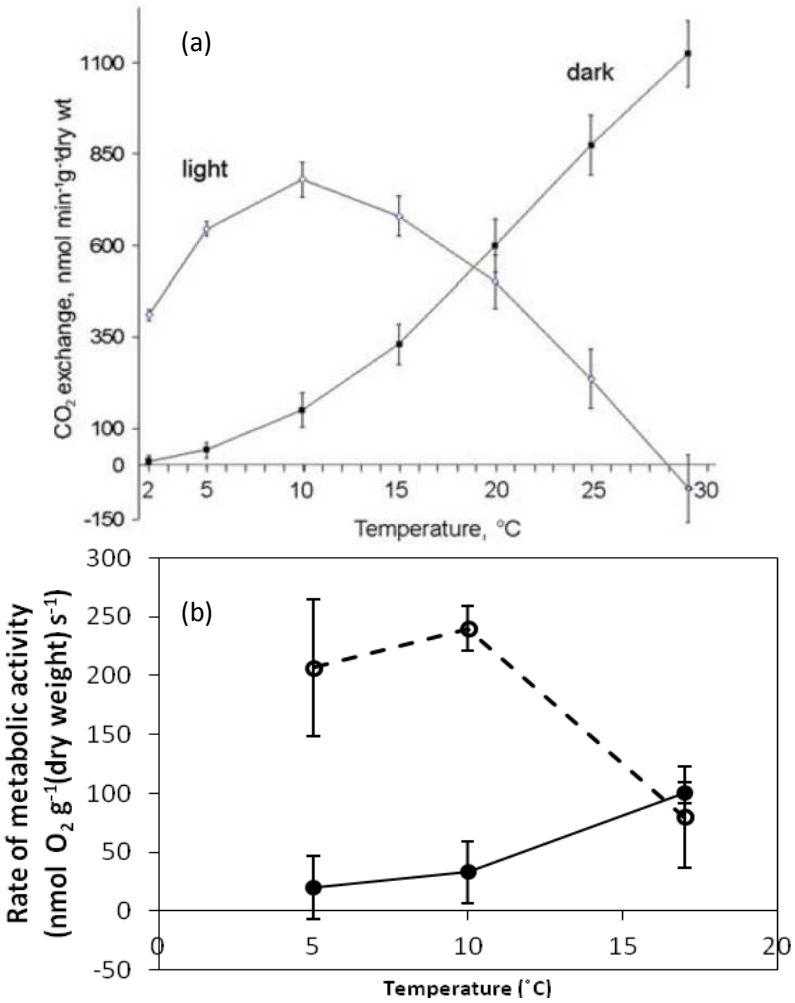

Figure 4.4.1: (a) Figure 1 from Aubert *et al.* (2007) illustrating the rate of CO_2 exchange of *X. elegans* thalli, at 500 µmol m^{-1} s^{-1} light intensity, at a range of temperatures. (b) Data for *C. stellaris* taken, in the present study, at 510 µmol m^{-1} s^{-1}, from the results in Section 4.3, rate of NP is depicted by open circles and DR by closed circles. Aubert *et al.* (2007) compared directly results from IRGA and oxygen electrode measurements, so the comparison here is justified.

It is worth noting here that in *C. mitis* and *C. rangiferina*, a Levene's test found that there was evidence that the size of the standard error of the data had significantly changed over time. It is evident in Figure 4.2.2 that the error bars became larger at lower temperatures. It may be possible therefore that the oxygen electrode apparatus is less precise at lower temperatures than at higher temperatures; a fact which should be considered in further studies.

4.4.2 The change of thallus wet weight over the course of the experiment

It is evident in the Figure 4.3.1 that hydration process did not provide a constant wet weight at which thalli could be measured; this is expected as previous studies have shown that the maximum wet weight of lichen thalli will be different each time they are hydrated (Seaward, 1976). Even though wet weight of lichen thalli is known to affect photosynthetic rate (*see Chapter 1*) a high atmospheric CO_2 concentration has been chosen so that wet weight was not a direct factor in determining the photosynthetic rates described above. However if the change in wet weight is directional and permanent this may be due to a loss of biomass (dry weight) during handling or storage. This would affect the final results as all photosynthetic rate data is presented as a function of the dry weight and a loss of biomass would mean that some photosynthetic rate data has been underestimated. As finding dry weight of a lichen thallus is damaging, and will lead to reduced photosynthetic rate, wet weight has been used to determine whether that has been any change in the dry weight of the samples which has been used to calculate the photosynthetic rate per gram of dry weight.

Importantly, linear regression analysis results indicated that the wet weight of *N. arcticum, C. mitis* and *C. stellaris* thalli did not significantly change over the course of the experiment and it can be assumed that the dry weight values obtained at the end of the experimental period are valid for all of the measurements obtained. It can be stated confidently that for these three species differences in metabolic rates, Figure 4.3.2, were not due to differences of sample biomass.

The mean wet weight of *C. rangiferina* samples maintained a significant reduction over the course of the month in which the experiment was conducted. An analysis of the wet weight of individual thalli revealed three samples which exhibited a distinct a permanent reduction, the results from these three species has been removed from the data. It is evident from this experiment that *C. rangiferina* was unsuitable for this series of studies and as such the species was not included in the long-term exposure analysis.

4.4.3 The effect of temperature on NP

As experiments have shown with other species, temperature was a significant factor in determining the rate of NP of all species. All species exhibited significantly reduced mean rates of NP at 17˚C, when compared to rates obtained at 10˚C and 5˚C (Figure 4.3.2). The reduction in NP evident at 17˚C is most likely due to the exponential increase in the rate of respiration which occurs with rising temperature (Figure 4.3.1). The relationship between rising temperature, which results in greater rates of respiration and reduced rate of NP in lichens, is evident in other studies with the same and different species of lichens (e.g. Aubert *et al*., 2007; Adams, 1971; Ino, 1985; Lange, 2002). Furthermore the increase in DR with increasing temperature, exhibited a similar pattern to how the light compensation point of photosynthesis increased with environmental warming. The effect of temperature on the light compensation point was the same as that found in *L. muralis* and was most likely caused by the changing rate of DR with temperature (Lange, 2002).

The optimum temperature for net photosynthesis exhibited some variation between species: in *C. rangiferina* 10˚C clearly yielded the highest rate of NP, *N. arcticum* thalli had an optimal temperature of 5˚C and *C. mitis* and *C. stellaris* the rate of NP was not significantly different between 5˚C and 10˚C when the light source was on (Figure 4.3.2). The low optimum temperatures are what would be expected of lichen species adapted to an arctic environment. The data presented in this study suggest that climatic warming could significantly reduce NP of arctic lichens.

The optimum temperatures of NP, found in this study, conflict with those found in other studies for some species. Sonesson *et al*. (1992) found the optimum temperature of NP in mid-summer adapted *N. arcticum* thalli, collected from an arctic heath environment, to be 10˚C, which was not the optimal temperature of the species (Figure 4.3.2 (a)). However, in thalli collected in September and October optimum temperature of photosynthesis is closer to 5˚C (Sonesson *et al.*, 1992). The disparity of the optimum temperature of photosynthesis, between lichen thalli adapted to different seasons of the year, is well documented (*Chapter 1*). In the present study *N. arcticum* thalli were stored at mid-summer temperatures and it would be expected that thalli would exhibit a similar optimum temperature of photosynthesis to that found in Sonsesson *et al.* (1992); potentially this difference could be explained by the phenotypic adaptation of thalli to specific ecotypes (Tretiach & Brown, 1995). *C. stellaris* thalli have also been shown to have an optimal temperature of NP at 20˚C, however this value was obtained from a North American population and the differences could be either genetic or due to phenotypic plasticity as *Cladina* lichens have been shown to exhibit high levels of variation in their response of NP to temperature between thalli from different geographical populations (Lechowicz, 1978; Macfarlane *et al.*, 1983).

The differences between the optimum temperatures of NP exhibited by lichen species in the study reviewed in this section, and other studies reviewing the same species, enhances the idea that lichen thalli exhibit photosynthetic differences between individuals from different populations. When studying the effects of environmental conditions on lichen species, thalli should be collected from a range of areas and the potential for phenotypic plasticity of samples should always be considered. In order to truly understand the potential of phenotypic adaptation in lichens more study on the subject is required and the effect of long-term exposure to environmental variables needs to be considered (Chapter 5).

4.4.4 The effect of light intensity on NP of lichens

Light intensity was shown to significantly affect the rate of NP in all species (Table 4.3.2). All species exhibited curve photosynthetic response to light intensity curves that have been found in other studies (Lange, 2002; Sonesson *et al.*, 1992). At 5˚C and 10˚C thalli exhibited higher levels of photoinhibition than at 17˚C, in all species, this can be seen clearly when considering rates of gross photosynthesis (GP), where at 680 µmol m^{-2} s^{-1} mean GP, of all species, was highest at 17˚C (Figure 4.3.4). This may be due to a similar relationship to that found in *L. muralis*, where the light use efficiency of photosynthesis increased at higher temperatures (Lange, 2002). The presence of a relationship, between temperature and the light efficiency of photosynthesis, would explain the presence of the significant interaction effect, between light intensity and temperature on NP, evident in all species studied except *C. mitis* (Table 4.3.2). If photosystems are photosynthesising in a more efficient manner they are less likely to experience oxidative damage associated with high light stress and will exhibit higher photosynthetic capacity (Lange, 2002). That peak GP in the *Cladina* species was achieved at 10˚C, however, is most likely due to the thermodynamics of enzymes, which are important in the photosynthetic process, where optimum yield is temperature-sensitive, and not the increase in light efficiency of photosynthesis (Brown & Kershaw, 1986).

4.5 Conclusions

It has been shown that the oxygen electrode apparatus is a viable method for measuring the response of NP and DR, of lichens, to changing climatic variables. However as *C. rangiferina* did not respond favourably to the experimental method the species has been excluded from further experimentation.

As expected both temperature and light intensity have been found to significantly affect the NP of the lichen species studied. Optimum temperatures of photosynthesis found in this study have been slightly lower than those observed in studies on the same species in other studies; it is hypothesised that the differences are due to phenotypic plasticity of lichens or genetic differences between populations from different continents. The significant interaction effect between light intensity and air temperature, on NP of lichens, can be explained by the increased light efficiency of photosynthesis found in other species.

The optimum temperature of photosynthesis of *Cladina* species in this experiment are marginally different to those found in other studies. Differences may be due to adaptation to the slightly different environment that the lichens in this study were collected from.

Chapter 5

The effect of long-term temperature regime on the photosynthetic rate of *N. arcticum*, *C. stellaris* and *C. mitis* thalli.

5.1 Introduction

It has been shown that environmental conditions can have an additively detrimental effect on lichens. For example the photosynthetic rate of *Lasallia pustulata* thalli, was significantly affected by the duration of irradiation, as opposed to the magnitude or the frequency of light intensity, to which the thalli were exposed; this effect could be applicable to other environmental conditions such as temperature (Bartak *et al.*, 2008). A study has shown that the effect temperature has on photosynthetic rate in lichens of the genus *Umbilicaria* changes according to the length of time which a thallus is exposed to a set temperature; importantly these changes occur over the course of a 24 hour period and at 14˚C, a temperature that would not normally be regarded as stressful (Larson, 1982). A longer term study, on *Cladina rangiferina*, illustrated that at higher temperatures (25˚-45˚C) net photosynthetic rate of the lichen thalli is increasingly more reduced the more extended the exposure period (Tegler and Kershaw, 1980). Lichens have also been shown to exhibit photosynthetic acclimation to variable air temperatures (*Chapter 1*). Potentially, lichens which are able to exhibit photosynthetic plasticity may be less affected by future climate change (Gunderson *et al.,* 2010). This experiment investigated whether the discrepancies, in the temperature at which maximal photosynthetic rate of lichen thalli is achieved, between *ex situ* controlled temperature experiments and *in situ* monitoring experiments, is in any way due to different effects of prolonged and short-term exposure to a set temperature.

In Chapter 1 evidence that the chlorophyll content of lichen thalli responds to environmental conditions was outlined. Previous studies have shown that such changes can occur over the course of a week (Brown and Kershaw, 1984; Sonesson *et al.*, 1992). It has also been shown that the growth rate and the condition, of lichen thalli, can be significantly influenced by external environmental factors. Under favourable conditions, lichen thalli experience an increased rate of growth, exhibit a reduced number of morphological anomalies and have a higher photobiont to mycobiont ratio (*see Chapter 1*). If the differences in photosynthetic rate of lichen thalli, due to the prolonged exposure to different temperatures, are due to the effect temperature has on the biomass of the lichen thalli, we could expect it to be evident in the weight of the thalli at the different times of measurement. It is hypothesised that the different environmental temperatures will result in different rates of growth

for the lichen samples, be they beneficial or detrimental, which would be evident in any changes of sample wet weight between the different measurement times.

This chapter provides a detailed report of a study that measured the response of NP, to air temperature at three different intervals, during a prolonged period of exposure. The report details: the method used to conduct the experiment, the data presenting the response of NP of the thalli to the experimental parameters, statistical test results which detail the factors that significantly affected NP and a discussion of the importance of the results.

5.2 Method

In order to allow comparisons with the previous experiment the method by which NP and DR rates were acquired was the same as described above. However, in this instance, when not being measured, samples were stored within climate-control chambers, set at specific climate regimens which more accurately mimicked the natural environment which the sample thalli were collected from; as opposed to chambers regulated at a constant temperature and light value.

The Fitotron growth chambers were set at three different regimes which resulted in daily average temperatures of 5˚, 10˚ and 15˚C. Each regime was a 24 hour diurnal fluctuation of temperature and light intensity, over the 24 hour period chamber temperature reached a maximum of 2.5˚C above the average temperature and a minimum of 2.5˚C below, light intensity was a 0 μmol m^{-1} s^{-1} for 10 hours of the cycle and at 700 μmol m^{-1} s^{-1} for 12 hours of the day (there were also two hours at 350 μmol m^{-1} s^{-1} between the 0 and 700 setting to simulate dusk at dawn). Figure 5.2.1 provides exact values for fluctuations in temperature and light intensity for each of the three regimens. The other factors which significantly affect lichen NP and growth were held at a constant value: relative humidity was set at 80% throughout the 'day/night' cycle and atmospheric CO_2 concentration was left ambient at ~380ppm (although this was not actively maintained). Samples were, during periods which they were not in a state prepared for measurement, hydrated daily and allowed to desiccate; this provided regular wet-active-time which reduces the effect of resaturation depression and abates sample degeneration due to lack of photosynthesis (Rogers, 1971). Samples were moved from one regime to another in sequence without a break as this reduces any degenerative effects of sudden climatic variation and is the most likely way thalli would encounter temperature changes *in situ* (Benedict *et al.*, 1990).

The regimens were designed to simulate ecologically significant periods of the year: mid-summer (considered to be peak-growing season for vascular plants within similar ecosystems), early spring and late summer/early autumn (potentially important periods for non-vascular productivity). Regimen climatic cycles were created in reference to the surface-temperature data presented in Figure 5.2.2; ground-surface temperature was chosen at it more accurately represent the temperature within which terricolous lichen inhabit.

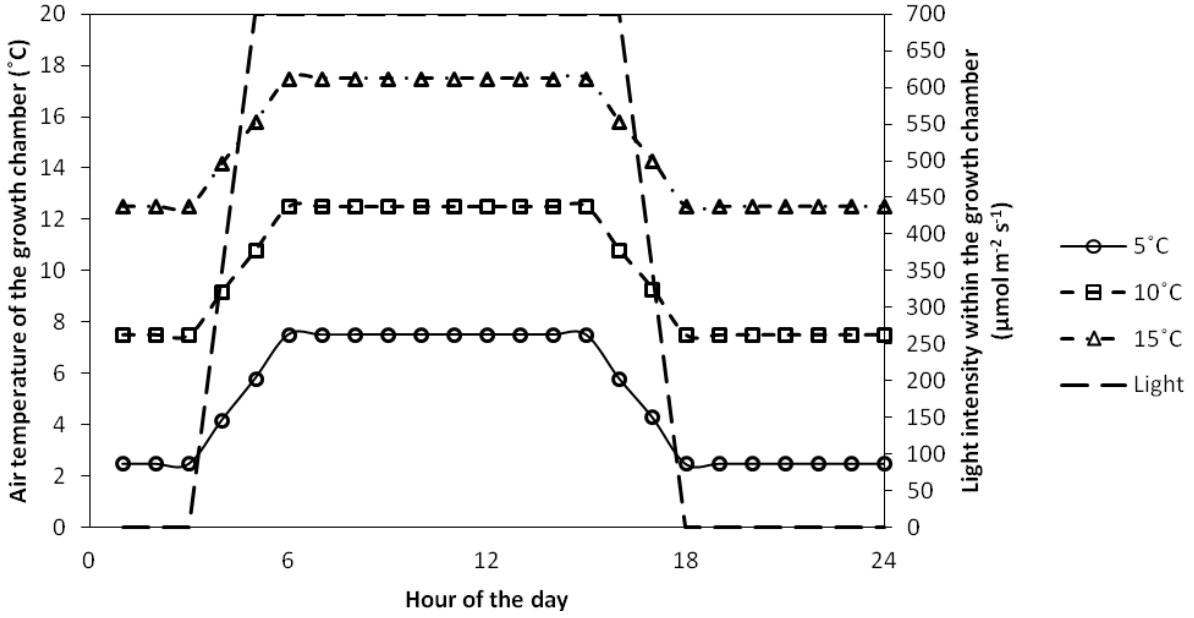

Figure 5.2.1: Graph indicating the temporal variation of the conditions within the storage chamber. The light intensity cycle was the same for every temperature regimen. Regimens are separated according to the average diurnal temperature (see legend).

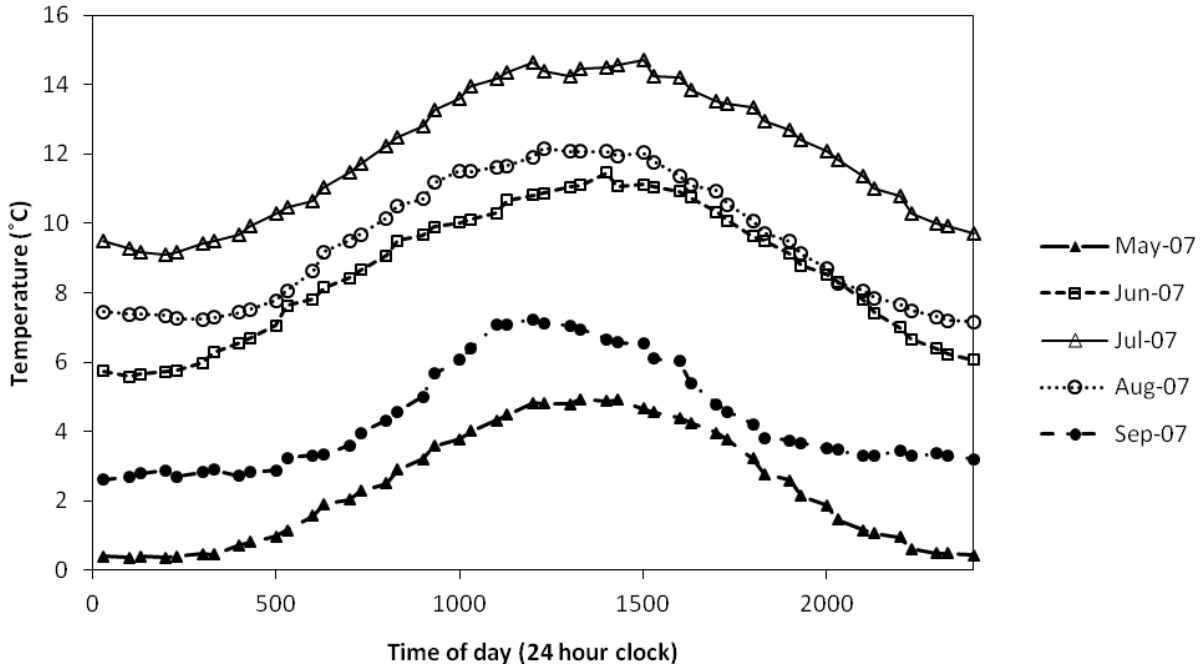

Figure 5.2.2: Average surface temperature at different times of day during different months of the year in Abisko, Sweden (68.37˚N, 18.69˚E), during the year 2007 (Baxter, unpublished). Maximum temperature during the year was achieved in July and was 24.88˚C. These data are corroborated by similar temperature during the year 2008 and from temperature data in Sonesson *et al.* (1992).

The environmental conditions within the 5˚C regime are equivalent to the surface-temperature changes which occur during May or September, at Abisko, Sweden. These months are significant as they represent periods where lichens are capable of growth but are outside of the peak season for vascular-plant primary productivity (Campioli *et al.*, 2009). The 10˚C regimen and corresponds to the ground surface temperatures of the early and late-summer months (August and June); a large proportion of vascular net primary productivity occurs during these months (Campioli *et al.*, 2009; Williams *et al.*, 2000). The 15˚C regimen represents the warmest part of July (as values are slightly higher than the mean temperature values for the whole of the month of July, for both 1988, 2007 and 2008) the purpose of this regimen is two-fold: first it represents the conditions during the peak growth-period for vascular plants and secondly it represents environmental conditions, which the lichen species studied will be subjected to, which would be prevalent for longer periods of the year under future climate warming predictions (Eliseev *et al.*, 2009; Campioli *et al*., 2009; Williams *et al*., 2000).

Initial photosynthetic rate measurements were made prior to storage and two sets of data were acquired during the six days that samples were stored within each regime; the first set was recorded after 24 hours storage under each specific regimen and the second set was taken after 144 hours (six days). Samples were prepared for measurement as per Chapter 3 .The method of oxygen electrode use outlined in Chapter 3 was also used in this experiment. All rate of NP measurements were taken at 340 μmol m^{-2} s^{-1} The wet weight of each thallus was recorded before and after placement within the electrode chamber. After the rate of NP and DR of a thallus was recorded thalli were replaced in the control-chamber and allowed to desiccate. NP and DR rate analysis was always performed with air temperature set at the average of the climatic setting the sample was stored under and at a light intensity of 510 μmol m^{-1} s^{-1}. Importantly, samples were always collected at the same time of day, to ensure climatic conditions at time of removal were a constant, this should have removed any possibility of the results being affected by the presence of a circadian rhythm. The second data set was taken after the samples had spent six days under a temperature regime; although thalli were prepared for measurement 24 hours before any rates were recorded. Six days was chosen as there are, to my knowledge, no studies of the rate of acclimation for these species. However, other studies have found significant levels of temperature adaptation after a week of transplanting *P. rufescens* thalli into a new temperature regime (Brown and Kershaw, 1984).

5.3 Results

5.3.1 Analysis of NP rate data without separation by species

The first analysis, presented here, considers all of the data, without weighting by species, in order to determine whether the response of each species was significantly different from the others. The aggregate data recorded, which represents the rate of evolution of O_2 for all species at all temperatures and after all lengths of storage, have been found to be normally distributed (Kolmogorov-Smirnov test: N= 270, Z= 1.164, *P*= 0.133 – not significant). Although a Levene's test has found that the results exhibit a significant heterogeneity of error variance ($F_{(26\ 243)} = 3.102$, $P < 0.001$ - significant) all group sizes are equal (N=30) and as such Equation 1 has been satisfied; therefore a three-way ANOVA analysis can be conducted. Importantly the analysis results indicate that mean rate of photosynthesis for some species was significantly dissimilar to other species ($F_{(2\ 243)}= 16.137$, $P< 0.001$). A post-hoc Tukey test revealed that *C. stellaris* mean photosynthetic rates were significantly different from both *N. arcticum* ($P< 0.001$) and *C. mitis* ($P< 0.001$), however *N.arcticum* and *C. mitis* means were not ($P= 0.877$). The difference between species is most evident in Figure 5.3.1, it is evident that *C. mitis* and *N.arcticum* had a similar magnitude of photosynthetic rate, while the rates for *C. stellaris* thalli were considerably greater, but all species maintained a similar reduction of mean photosynthetic rate as temperature increased.

The analysis results suggest that there was a significant interaction effect of species and length of storage on the mean rates of photosynthesis ($F_{(4\ 243)}= 6.544$, $P< 0.001$); even though length of storage does not seem to be a significant factor in itself ($F_{(2\ 243)}= 2.143$, $P=0.119$). Temperature was also a significant factor of the photosynthetic rate of thalli and unlike the previous experiment mean photosynthetic rates are found to be significantly different between all three temperatures. The difference between temperature settings is illustrated in Figures 5.3.1 and 5.3.2 where maximal rates of photosynthesis are achieved at 5˚C in all species and at each of the three storage lengths and decrease linearly as temperature rises. The effect of temperature on photosynthetic rate of thalli was significant ($F_{(2\ 243)}= 64.432$, $P<0.001$) and species did not exhibit significantly different responses to temperature to each other($F_{(4\ 243)}= 1.497$, P=0.204).

Photosynthetic rate of thalli, for all species data combined, did not change significantly with increased time stored at a set temperature at any of the temperatures measured ($F_{(4\ 243)}= 1.267$, P= 0.284).

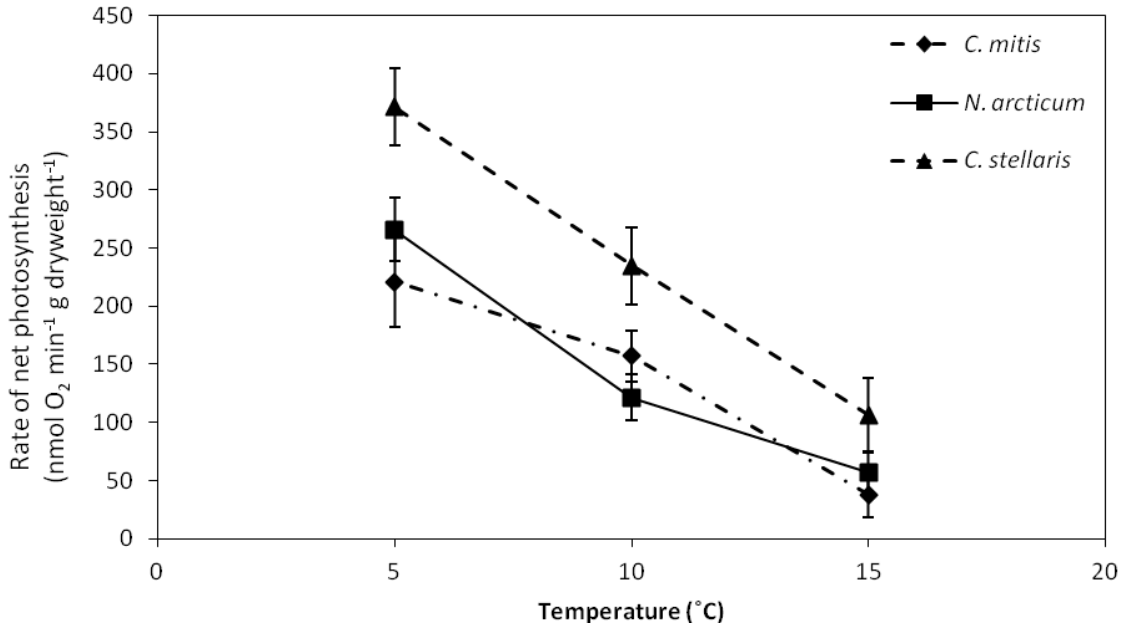

Figure 5.3.1: Graph depicting the mean photosynthetic rate of thalli, for each species, at different temperatures. Mean rate was taken from all storage length data and each point represents the mean photosynthetic rate of 30 thalli. Error bars indicate +/- one standard error.

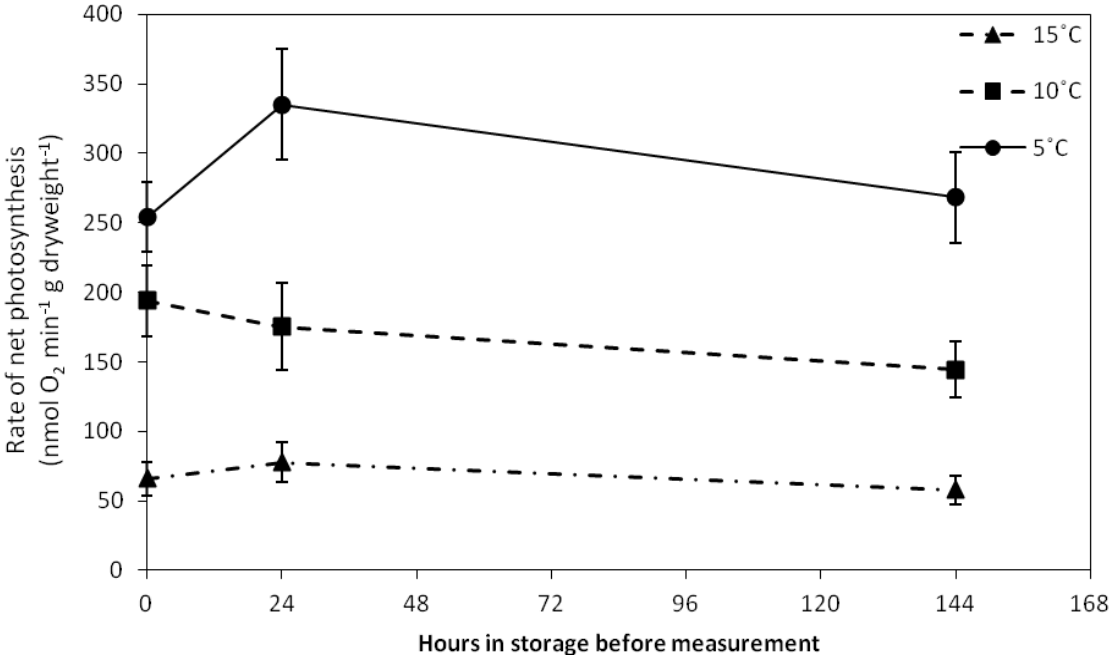

Figure 5.3.2: Graph depicting the mean photosynthetic rate of thalli at different temperatures over the course of the storage period. Mean rate was taken from all species data and each point represents the mean photosynthetic rate of 30 thalli. Error bars indicate +/- one standard error.

5.3.2 Individual analysis of each species' response to storage time

Because there is evidence that there was a significant interaction between the effect of species and the effect of length of storage time had on photosynthetic rates of lichen in the three-way ANOVA results in this section ($F_{(4\ 243)}$= 6,544, P<0.001), it is possible that, although length of storage time was an insignificant factor in the three-way ANOVA, it may be significant when species data are considered individually (Pallant, 2007). In order to test for such an effect, a two-way ANOVA analysis was performed where the effect of the independent factors or air temperature and length of storage time were analysed for each species separately.

Results of the two-way ANOVA, Kolmogorov-Smirnov and Levene's tests of the effect of temperature and length of storage time on NP of *N. arcticum*, *C. mitis* and *C. stellaris*, are described in Table 5.3.1. In all cases where a Levene's homogeneity of variance test was significant, N of all groups was the same and Equation 2 was satisfied, and two-way ANOVA results could be analysed under standard conditions.

Nephroma arcticum:

The results of the two-way ANOVA test imply that both air temperature and length of time at each temperature significantly affected the rate of photosynthesis of *N. arcticum* thalli. In this species there was no evidence of a significant interaction effect between the two factors. The lack of interaction is evident in Figure 5.3.3 (a), where the shape of the response to time at a given temperature was similar at all temperatures. A post-hoc Tukey test suggests that the mean rate of photosynthesis at 5˚C was significantly larger than at the other two temperatures (P< 0.001 for both comparisons), however the 10˚ and 15˚C groups were not significantly different from each other (P=0.097). Furthermore Tukey test results suggest that there was a significant difference in the photosynthetic rate results obtained after 144 hours at a given temperature when compared to data obtained at 0 (P= 0.043) and 24 (P= 0.020) hours after storage. Intriguingly however the rate of NP increased significantly between the 24 hours and 144 hours groups, at 10˚ and 15˚C, which reduced the difference in photosynthetic rate evident at 0 hours, between these temperature groups and the 5˚C group.

Cladina mitis:

The two-way ANOVA results suggest that temperature was a significant factor in the rate of photosynthesis of *C. mitis* thalli, however the mean rate of photosynthesis did not significantly change with increasing length of storage time. There is no evidence that there was a significant interaction between the two factors in this species. Post-hoc Tukey test results suggest that the mean rate of photosynthesis of the 15˚C groups was significantly lower than the photosynthetic rate data of the other two temperature groups (P= 0.008 in comparison with 10˚C. The two-way ANOVA analysis results are evident in Figure 5.3.3 (b); the 5˚ and 10˚C responses to length of time at a given temperature vary in a fashion similar to each other while at 15˚C the rate of photosynthesis of *C. mitis* thalli was depressed at 0 and 144 hours. The graph does seem to suggest that after 144 hours at 15˚C was even more suppressed than at both 0 and 24 hours even though the reduction in photosynthetic rate has not been found to be significant.

Cladina stellaris:

The two-way ANOVA test results suggest that both air temperature and the length of time storage at a given temperature significantly affected the rate of photosynthesis of *C. stellaris* thalli. There was a significant interaction effect between the two factors. The interaction effect is visible in Figure 5.3.3 (c), where at there was a notably greater reduction in the mean rate of photosynthesis between the 24 hours data collection period and the 144 hours collection period, at 10˚C when compared to the other temperatures. A post-hoc Tukey test suggests that the mean rate of photosynthesis of all temperature groups were significantly different from each other (P< 0.001 for all group comparisons). Tukey results imply that the mean rates of photosynthesis of *C. stellaris* thalli were significantly different between the 24 hours and 0 (P= 0.013) and 144 (P< 0.001) hours, but the 0 and 144 hours groups did not differ significantly (P= 0.279).

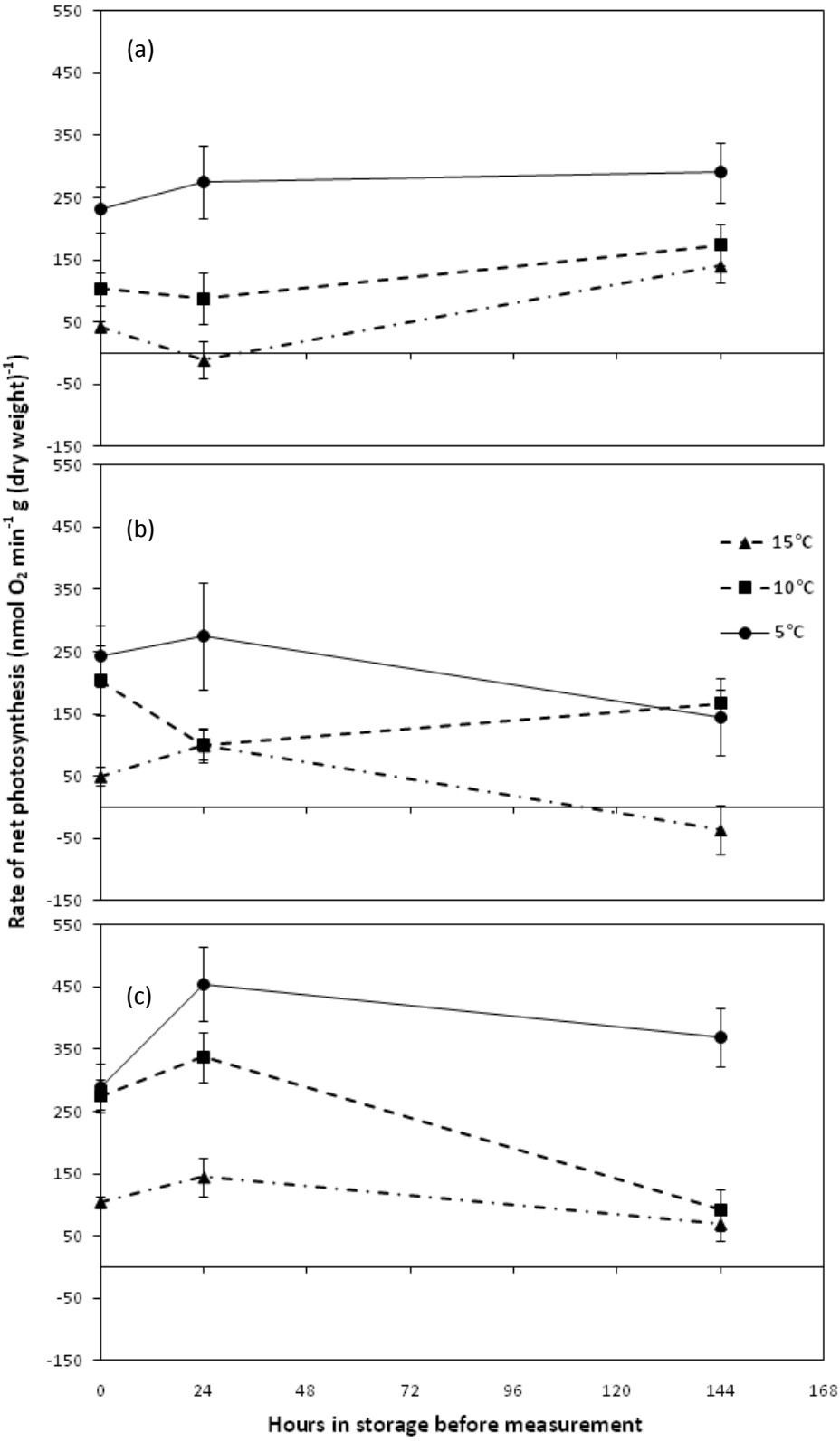

Figure 5.3.3: The change in mean photosynthetic rate over the course of the experiment at each set temperature for each of the three species: (a) *N. arcticum*, (b) *C. mitis* and (c) *C. stellaris*. Mean rates are calculated from 10 sample thalli and error bars are calculated as +/- one standard error. Legend temperatures indicate mean air temperature of storage regime.

Table 5.3.1: Results of two-way ANOVA testing for the significance of the effect of air temperature and storage time on mean photosynthetic rate of all three lichen species, and for the presence of a significant interaction effect between the two independent factors. Significant results are marked with an *. Tests of data normality and error homogeneity are also displayed.

	Kolmogorov-Smirnov test			Levene's homogeneity of variance test			Two-way ANOVA			
Species	N	Z value	*P* value	df	F value	*P* value	Independent factor	df	F value	*P* value
N.arcticum	90	0.614	0.845	8, 81	2.781	0.009*	Air temperature	2, 81	24.820	< 0.001*
							Storage time	2, 81	4.528	0.014*
							Interaction effect	4, 81	0.884	0.477
C. mitis	90	0.927	0.356	8, 81	4.213	< 0.001*	Air temperature	2, 81	11.507	< 0.001*
							Storage time	2, 81	2.204	0.117
							Interaction effect	4, 81	1.598	0.183
C. stellaris	90	0.775	0.585	8, 81	2.066	0.049*	Air temperature	2, 81	37.864	< 0.001*
							Storage time	2, 81	10.248	< 0.001*
							Interaction effect	6,	3.681	0.008*

5.3.3. Wet weight change over time

In order to determine whether the wet weights of samples have significantly varied over the course of the experiment, and whether any significant change in weight was due to the effect of temperature, a repeated measures analysis was conducted. Species have been analysed individually as the three-way ANOVA results suggested that the responses to the experiment of each species were significantly different from one another. Results of the repeated-measures analysis are presented in Table 5.3.2.

A Levene's test found that the error variances were not significantly dissimilar for the wet weight data obtained at 24 and 144 hours for all species. A repeated-measures analysis discerned that time within the climate regime did not significantly affect the mean wet weight of sample thalli of any species.

N. arcticum:

Table 5.3.2 indicates that neither time in the growth chamber nor temperature of the climate regime significantly affected the mean wet weight of sample thalli over the course of the experiment. Furthermore there was no indication that there was an interaction between the two factors and the effect of time was not significant under any temperature regime. As wet weight did not significantly change of the course of the experiment it has been assumed that dry weights at accurate for all measurements and that differences between temperature regimes can not be explained by changes in the biomass of samples.

C. mitis:

The repeated measures analysis results suggest that there was not a significant change in the wet weight of the *C. mitis* thalli between the 24 and 144 hours measurements, however there was evidence of a significant interaction effect between time in storage and temperature. Furthermore the wet weights were not significantly different at each temperature suggesting that any effect of temperature on the rate of photosynthesis of *C. mitis* thalli, found in the two-way ANOVA described above, is not due to changes in the weight of sample thalli during storage. In Figure 5.3.4 (b) there is evidence of an interaction between the effect time left in storage and the effect temperature had on the wet weight of the sample thalli, at 15˚C there is a clear reduction in wet weight and then as

temperature decreases the mean wet weight of *C. mitis* thalli increases between the two time periods; this trend is not permanent as the thalli return to a the same mean weight as was achieved at the beginning of the experiment.

C. stellaris:

The repeated measures analysis results suggest that although time did not significantly affect the mean wet weight of sample thalli, within 144 hours under each climate regime, the *P*-value (0.051) indicates that if samples had been left in the growth chamber for a longer period of time, time in the growth chamber could have been a significant factor in determining the wet weight of sample thalli. There was no evidence of a significant interaction effect between time in storage and temperature. Furthermore the wet weights were not significantly different at each temperature suggesting that any effect of temperature on the rate of photosynthesis of *C. steallaris* thalli, found in the two-way ANOVA described above, is not due to changes in the weight of sample thalli during storage. Figure 5.3.4 (c) does not strongly corroborate the results of the repeated measures analysis as there is evidence that mean wet weight of the lichen thalli consistently increased between the 24 and 144 hour measurements at each temperature, furthermore mean wet weight seems to increase as temperature is lowered; perhaps a larger N would provide greater resolution for this species.

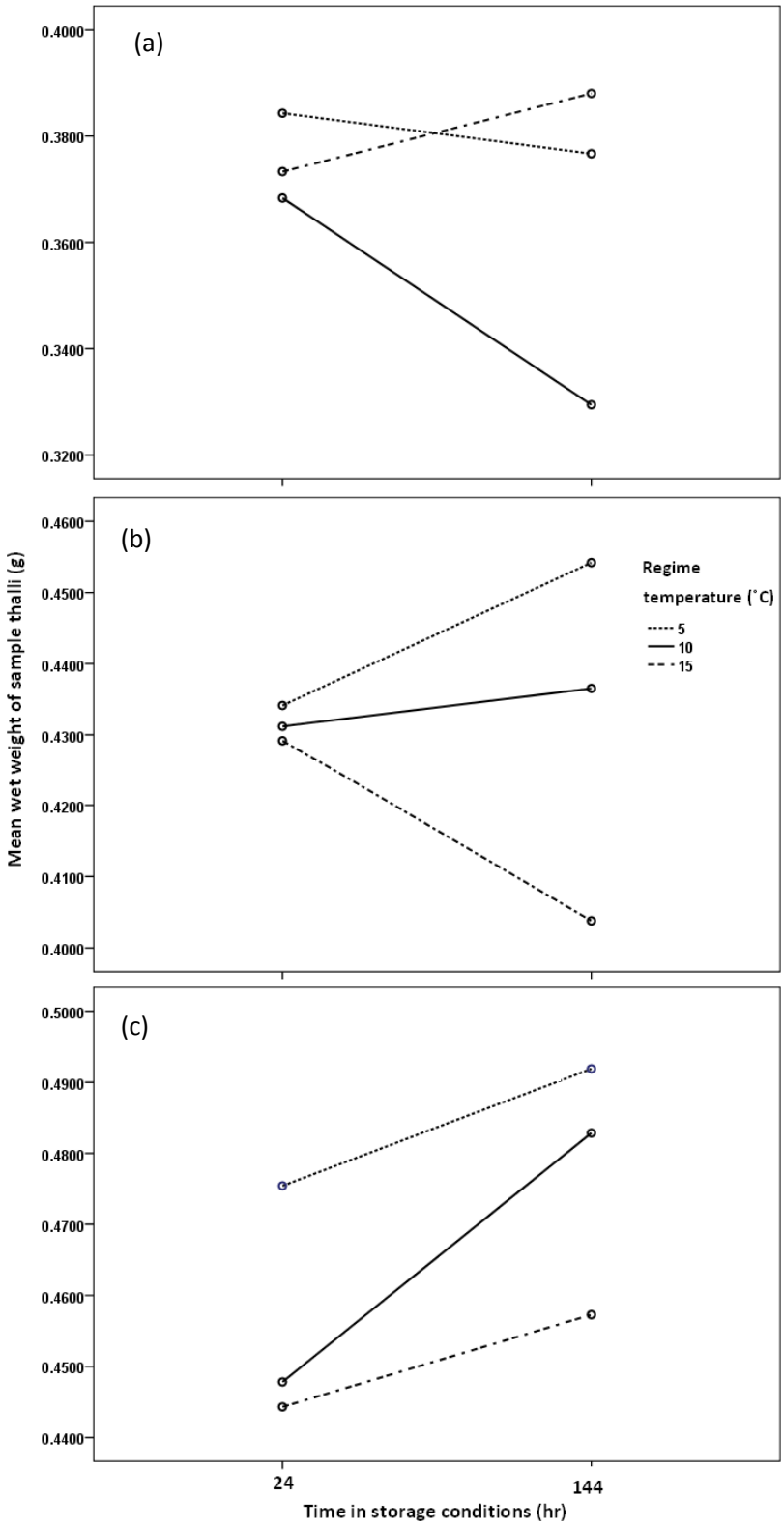

Figure 5.3.4: Mean wet weight of sample thalli, at 24 hours and 144 hours in each temperature regime, prior to measurement in oxygen electrode apparatus. Legends separates temperature regime according to mean temperature of each regime. Species depicted are: (a) *N. arcticum*, (b) *C. mitis* and (c) *C. stellaris*. Error bars have been excluded to avoid reduced clarity.

Table 5.3.2: Results of repeated measures analysis, testing for the significance of the effect of time in storage and temperature of storage on mean photosynthetic rate of all three lichen species, and for the presence of a significant interaction effect between the two independent factors. Significant results are marked with an *. Tests of data normality and error homogeneity are also displayed.

	Levene's homogeneity of variance test 24 hour data			Levene's homogeneity of variance test 144 hour data			Repeated measures test			
Species	df	F value	*P* value	df	F value	*P* value	Independent factor	df	F value	*P* value
N.arcticum	2, 27	0.138	0.872	2, 27	1.529	0.235	Time	1, 27	0.744	0.396
							Temperature regime	2, 27	1.148	0.332
							Interaction effect	2, 27	1.600	0.220
C. mitis	2, 27	0.843	0.441	2, 27	0.726	0.493	Time	1, 27	0.000	0.999
							Temperature regime	2, 27	0.828	0.448
							Interaction effect	2,27	3.793	0.035*
C. stellaris	2, 27	1.386	0.267	2, 27	0.010	0.990	Time	1, 27	4.156	0.051
							Temperature regime	2, 27	0.845	0.441
							Interaction effect	2, 27	0.422	0.660

5.4 Discussion

The air temperature of the environment in which a lichen thallus is found has been shown to significantly affect the rate of NP of the thallus (*Chapters 4 and 1*). It is also known that the length of time that a lichen thallus is exposed to an environmental factor that determines rate of NP significantly alters the response of a thallus to that factor (*see Chapter 1*). However lichens exhibit a high level of species variability and it is important to consider each species individually. This section discusses the response of the mean NP and mean wet weight, of each species, to variable air temperature and how this response changed over the course of 6 days of exposure.

5.4.1 The effect of storage regime on biomass of lichen thalli

Time under the storage regime used in the experiment reported in this chapter, did not significantly affect the wet weight of sample thalli of any species. There was also no significant difference between the wet rates of sample thalli under the different temperature regimes. From the repeated measures test results, it can be assumed that the differences in mean photosynthetic rate of lichens at different temperatures was not due fluctuations in sample biomass. However *C. stellaris* exhibited a general increase in wet weight over the course of the experiment, that was almost significant, and *C. mitis* exhibited a significant change in wet weight during the 15˚C storage; it is possible that if less variable weight analysis was conducted or if storage had continued for a longer period, then the storage method may have significantly affected the biomass of the thalli of these species. Due to the inherent variability of wet weight measurements (*see Chapter 4*) it would be preferable if future analysis could be performed by looking at change in dry weight of samples, as dry weight is more closely associated with biomass. Anecdotally *C. stellaris* thalli seemed to become greener as the storage temperature decreased, this may be associated with an increase in chlorophyll content, it would be prudent in future experiments, on the effect of long term exposure to different environments on lichens, to perform periodic chlorophyll content analysis on samples. This could not be performed in this experiment as current methods of measuring chlorophyll content are destructive and there were not enough of the sample thalli to complete a full analysis.

5.4.2 The effect of long term exposure to different temperatures on NP of lichens

From the three-way ANOVA results it can be seen that mean rate of NP of the three species of lichens studied significantly increased with reduced temperature, this was similar to the results

obtained in the experiment reported in Chapter 4. When species are not considered individually, there was not a significant change in the rate of NP between 0 and 144 hours under each climate regime, but the results are different when considering each species individually.

The NP of *N. arcticum* thalli exhibited a very similar response of temperature in Figure 5.3.3 (a) as it did in 4.3.2 (a). The optimum temperature of NP was 5˚C and the mean rate of NP was not significantly different at 10˚C and 15˚C in both experiments; this general response was evident regardless of the length of time which thalli were exposed to each temperature regime. However after 24 hours under the storage conditions used (described in the methods of this Chapter), the mean NP of thalli significantly increased at 10˚C and 15˚C (Figure 5.3.3). The significant increase in mean rate of NP at 15˚C was unexpected, as temperature and hydration conditions were the same as they had been under previous storage conditions, but could be attributed to thalli photosynthetically adapting to the new temperature regimes as *N. arcticum* thalli are known to be quite phenotypically plastic (Sonesson *et al*., 1992). As the hydration conditions, of sample thalli, were exactly the same under each of the temperature regimes (and the same as the storage conditions previous to this experiment) it must be assumed that all differences are due to either air temperature or light regime. A plausible explanation of why NP of thalli generally increased as the temperature of storage decreased is because samples stored at lower temperatures may have longer periods of active wet time. It has been shown that active wet time is an important factor in determining the NP of lichens and thalli that are provided with more active wet time are better able to adapt at adverse conditions (*see Chapter 1*). It is also considered to be a general rule that lichens, as they are poikilohydric organisms, lose thallus water at a greater rate in higher temperatures and under periods of extended light exposure (Nash, 1996). It is possible that under the extended storage conditions *N. arcticum* thalli were able to achieve greater periods of wet active time, resulting in thalli which were more metabolically active after 144 hours, as the introduction of a diurnal light cycle reduced the rate of water loss of samples, compared to the rate of water loss under 24 hour light conditions. These results suggest that day length may be an important factor in process by which lichens photosynthetically adapt to new temperature regimes.

C. mitis thalli did not exhibit a significant change in the mean NP of sample thalli between the different storage periods at all temperatures (Table 5.3.1). Temperature response of mean NP was very similar at 0 and 144 hours, importantly there was no significant difference between the mean NP of thalli at 10˚C and at 5˚C as was evident in this species in Chapter 4 (Table 4.3.2), but at 15˚C (as with 17˚C in Chapter 4) mean NP of thalli was significantly suppressed. These results suggest that the rate of NP of *C. mitis* thalli is less temperature dependant, under optimal hydration conditions, than

C. stellaris (where all temperatures were significantly different from each other); this is to be expected as *C. mitis* is found in a more exposed environment where air temperature is more stochastic (*see Chapter 2*) and thalli could increase their active wet time by being achieving NP over a range of temperatures. Although it was not significant, Figure 5.3.3 (b) suggests that after 144 hours under the 15˚C temperature regime the mean rate of NP of *C. mitis* was reduced when compared to rate at 0 hours. The depressed mean NP could be linked to the significant reduction in mean wet weight of sample thalli exhibited at 15˚C between 0 and 144 hours. Higher temperatures may have resulted in sample thalli losing thallus water at significantly greater rate, than at lower temperatures, at could have lead to samples having a reduced thallus water content, which has been shown to reduce NP of thalli. As the NP response to temperature of this species did not significantly alter over the 6 day storage period, it has been assumed that thalli did not photosynthetically adapt or exhibit any phenotypic plasticity within this time constraint, this was unexpected as *C. mitis* has been shown to exhibit high levels of photosynthetic plasticity *in situ* (Lechowicz and Adams, 1974).

The length of time which *C. stellaris* thalli were exposed to a temperature regime has been shown to significantly affect the response to mean NP (Table5.3.1). Furthermore, as there was evidence of a significant interaction effect between time of storage and air temperature of the regime, it can be concluded that the temperature of the regime significantly affected how NP of thalli would respond to the length of exposure to each temperature. At 15˚C mean NP of thalli seemed constant with no change over the course of the storage time; NP was depressed in a similar fashion at 15˚C in this chapter as it was at 17˚C in Chapter 4. The mean rate of thallus NP in this species at 5˚C was significantly higher, than at the higher air temperatures, at both 24 and 144 hours of storage; this is different to the results found in Chapter 4 where mean rate of NP at 5˚C is statistically indistinguishable to the mean rate of NP at 10˚C. The NP of thalli of *C. stellaris* at 10˚C exhibited a reduction between 0 and 144 hours, this could be evidence of a theory suggested in Nash (1996) that the rate of NP obtained, at higher temperatures, in short-term temperature experiments is higher than can be realistically maintained. It is also possible that the reduction in NP, between 0 and 144 hours at 10˚C is due to the reversible reduction in photosystem activity, thought to be due to a shortening of the day length, found in North American populations of *C. stellaris* and *C. rangiferina*; although this is supposed to be coupled with a reduction in air temperature and NP is not reduced at 5˚C (MacFarlane *et al.*, 1983). These results suggest that our understanding of what causes the uncoupling of photosystems in reindeer lichens is very poor. The evident optimum temperature of photosynthesis over a long period of time, at 5˚C in this species is very different to results found in

other experiments, where the optimum temperature of photosynthesis is 10-15˚C (Ino, 1985; MacFarlane *et al.*, 1983).

5.5 Conclusion

From the data it can be concluded that the length of time that a lichen thallus is exposed to an air temperature, significantly influences how the rate of NP is affected by air temperature. There however no distinct patterns in how the length of exposure affects rate of NP: *N. arcticum* exhibited a significant increase, after six days storage, in mean NP at 10˚C and 15˚C, *C. stellaris* exhibited a significant reduction at 10˚C, after six days storage and the rate of NP of *C. mitis* was not significant affected by length of time exposed to an air temperature. The process by which the length of time a thallus was exposed to a temperature affects NP was most caused by different processes that are not well understood. It can be concluded that temperature did not affect the wet weight of samples prior to measurement and as such hydration status during measurement cannot be used to explain the difference exhibited in mean NP at different temperatures.

Species exhibit individual responses to temperature that are similar to those found in Chapter 4 and that can be linked to the niche environment in which the species is found. A full analysis of how each species responded to temperature from the results of both chapters will be performed in Chapter 6.

Chapter 6

Discussion of results obtained over the course of the study period

6.1 The individuality of species response to the variation of external environmental factors of metabolic activity

It has been shown that the response of lichen thalli to their abiotic environment is species specific (Figure 6.1.1; Ino, 1985). In many cases, the response of photosynthetic rate or the rate of dark respiration of a species can be related to the environment in which a species exists. A comprehensive study of fruiticose and foliose lichens has shown that the optimum temperature of photosynthesis of a species significantly increases as the latitude, at which the lichen species is commonly found, decreases (Lechowicz, 1982). In a similar fashion, many of the differences, between each lichen species, of the response of NP and DR to temperature and light intensity, found in the experiments performed in Chapters 4 & 5, can be explained by differences in the climatic conditions of the natural habitat of each species.

If a pattern can be found by which the response of metabolic processes within lichen thalli, to changes in their abiotic environment, can be related to the ecological and morphological characteristics of a species, then modelling both the ecological response of species to future warming and the cyclical change of seasonal climatic conditions could be modelled more efficiently.

6.1.1 Response of thalli to temperature

In order to compare the effect temperature on lichen thalli data has been compiled from Chapters 4 & 5. Chapter 4 data provide mean DR values and mean NP values, at 340µmol m^{-2} s^{-1} light intensity, prior to sample treatment detailed in Chapter 5, Chapter 5 data are those recorded after 6 days storage under a given temperature regime. The effects of temperature on dark respiration and net photosynthesis, after both short and long-term temperature exposure are presented in Figure 6.1.1.

N. arcticum thalli exhibited a very different response of rate of NP to temperature, to the *Cladina* species (Figure 6.1.1), which is to be expected as *N. arcticum* thalli are found in a much more shaded habitat where the forest floor is sheltered from irradiance and atmospheric perturbations by vascular plant biomass, interception of PAR will result in a cooler and reduced air flow in a less variable environment than a non-sheltered habitat (Matlack, 1993). The downward curving trend of the rate of DR exhibited by *N. arcticum*, as temperature increased, is similar to the curved trend of

the cold adapted species *Umbilicaria decussata* (Ino, 1985). *U. decussata* is also a foliose lichen species that has a high abundance on North-facing slopes in the continental Antarctic and potentially has a similar ecology to *N. arcticum* (Krzewicka and Smykla, 2004). The response of photosynthetic rate to temperature, in both *U. dicussata* and *N. arcticum,* could be described as cold-adapted; with the highest rate of NP being achieved at 5˚C and a curvilinear reduction of NP as temperature increased (Ino, 1985). As the other foliose species tested in Ino (1985), also exhibited cold adapted responses to temperature, it could be hypothesised that sub-Arctic foliose species are more likely to exhibit this temperature response pattern. This is corroborated by conclusions made by Showman (1979), where morphology as opposed to ecology of lichens determines whether they are susceptible to temperature damage. However the fuiticose species studied did not exhibit any conformity of response of NP to temperature and a more comprehensive study of the effect of thallus morphology on the response of lichens to their abiotic environment is required, using species with a range of morphologies from different ecosystems.

Of the *Cladina* species *C. rangiferina* exhibited a markedly different response, of NP to temperature, to the other species (Figure 6.1.1). In the results obtained in Chapter 4, *C. rangiferina* exhibited a distinctly convex curved response; this pattern is described as non-adapted curve, exhibited by generalist species, and is exhibited by *C. rangiferina* thalli from Japan (Ino, 1985). The results suggest that *C. rangiferina* is adapted to warmer temperatures than the other reindeer lichens, which may be indicative of the more sheltered environment that the species is typically found in (Lechowicz and Adams, 1974). From the results in Chapter 4, both *C. mitis* and *C stellaris*, exhibited slightly convex responses to temperature, where there was no significant difference between NP at 5˚ and 10˚C with NP decreasing as temperature was increased past 10˚C. However, after 144 hours at each temperature, the response of NP to temperature of *C. stellaris* changed exhibiting a cold-adapted response which was similar to the response of *N. arcticum*, while *C. mitis* thalli were not significantly affected. As *C. stellaris* occupies the most xeric and exposed, i.e. coldest, habitat of the reindeer lichens studied, the significant reduction of NP at 10˚C could have due to adaption to colder temperatures, or extended periods of hydration at high temperatures, which has been shown to cause high levels of oxidative damage (Figure 6.1.1) (Kershaw *et al.*, 1983; Minibayeva and Beckett, 2001; Nash, 1996). Of the four species, the NP of *C. mitis* was the least affected by temperature, where mean thallus NP exhibited no significant difference between NP at 5˚C and at 10˚C, in all measurements (Figure 5.3.3 (b)). This kind of response is expected of an early colonising species that would be exposed to a wide range of temperatures; however it was expected that the species would exhibit some photosynthetic plasticity when adapting to lower temperatures (Lechowicz and Adams,

1974). The response of fruiticose lichen species to temperature has been shown to be complex, from the results in this report, and is dependant not only upon the climatic regime the sample thalli were exposed to prior to measurement, but also on the geographical location of the sample population.

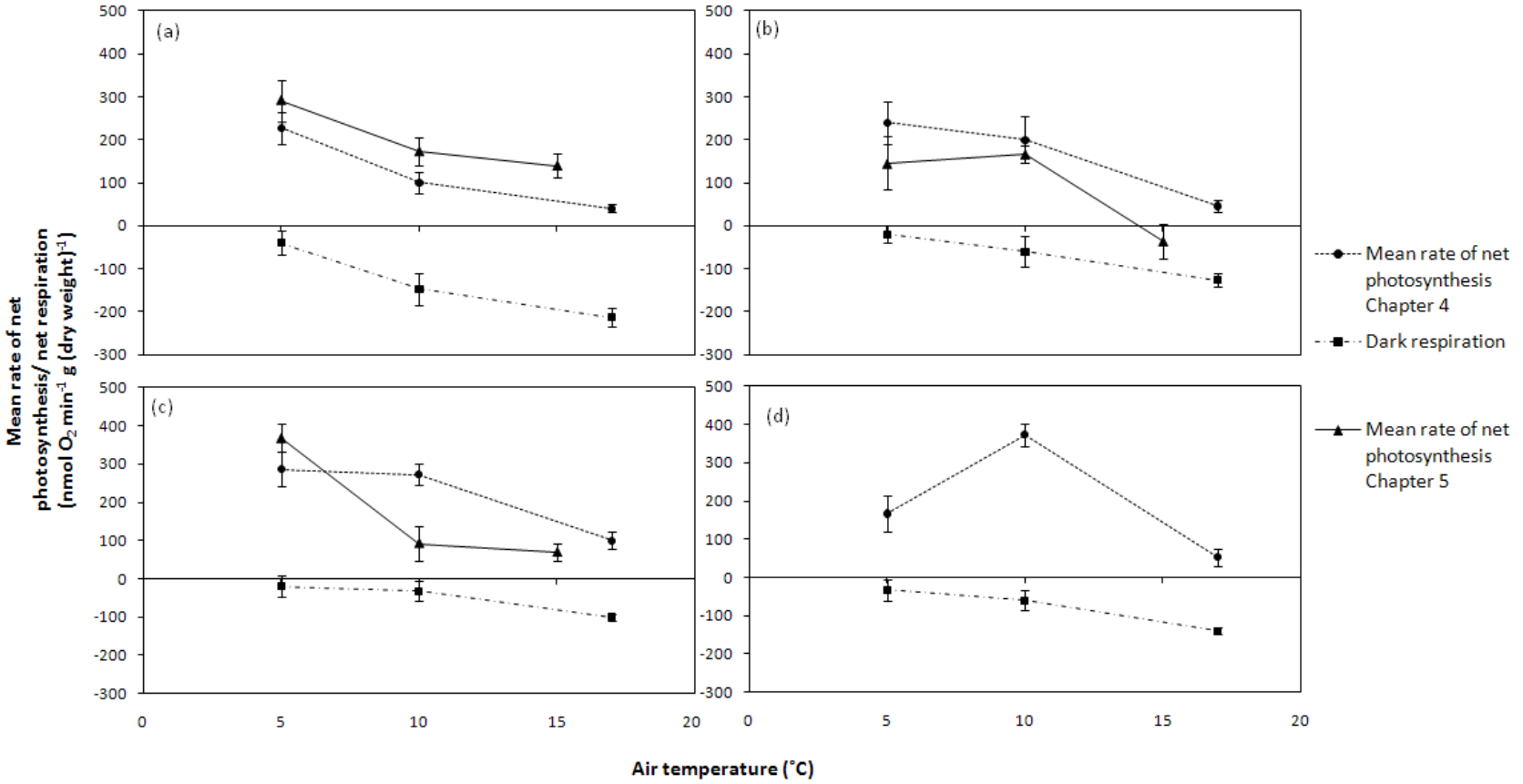

Figure 6.1.1: Mean NP (at 340µmol m^{-2} s^{-1} light intensity) and DR of all four lichen species at a range of air temperatures. Data are formulated from results from both Chapters 4 & 5, Chapter 4 data provide mean DR values and mean NP values prior to sample treatment detailed in Chapter 5, Chapter 5 data are those recorded after 6 days storage under a given temperature regime. Graphs are split by species: (a) *N.arcticum*, (b) *C. mitis*, (c) *C. stellaris* and (d) *C. rangiferina.* Error bars indicate +/- one standard error.

6.1.2 Response of NP to light intensity

In order to compare the relative effects of variable light intensity across species, the percentage reduction of photosynthesis has been calculated and is present in Table 6.1.1. Percentage reduction of NP is calculated as the mean NP at any given temperature light intensity scenario divided by the maximum NP obtained at the same temperature multiplied by 100.

Sample thalli of *C. stellaris*, the least shade tolerant species studied, generally exhibited the most reduced levels of photoinhibition of the four lichen species studied, at 510 µmol m^{-2} s^{-1} light intensity (Table 6.6.1). This was expected as the levels of photoinhibition, exhibited by thalli of other species of lichens, have been shown to be higher in species which typically inhabit more shaded niches (Manrique *et al.*, 1993). Unexpectedly, however, *N. arcticum*, a species which typically comprises an important part of the undergrowth in wooded, sub-Arctic heaths (Figure 2.1.1), exhibited no photoinhibition at 17˚ and 10˚C, at 510 µmol m^{-2} s^{-1} light intensity and lower levels than all other species at these temperatures at 680 µmol m^{-2} s^{-1} light intensity. Although this response is unexpected the values are clearly very similar to those obtained from summer adapted alpine *N. arcticum* thalli in Sonesson *et al.* (1992).

The response of lichen species to light intensity and how it is related to species ecology is further complicated when the light compensation points of photosynthesis are analysed. The two shade tolerant species, *N. arcticum* and *C. mitis*, exhibited higher light compensation points than the slightly shaded tolerant *C. rangiferina* thalli which in turn was higher than the least shade tolerant species, *C. stellaris* (Table 4.3.3) (Arseneault *et al.*, 1997). It would be expected that highly shade adapted species would be able to most take advantage of opportunistic light availability and would exhibit higher rates of NP at lower light intensities; which manifests as lower light compensation of photosynthesis points (Manrique *et al.*, 1993; Tretiach and Brown, 1995). The unorthodox light compensation points obtained may be due to species losing shade adaptive characteristics (such as high chlorophyll content) during storage.

Table 6.1.1: The percentage reduction of mean maximum thallus NP, for each temperature, at the two highest light intensities studied, for each of the four lichen species.

Species	Temperature	% reduction of NP at 510 µmol m^{-2} s^{-1}	% reduction of NP at 680 µmol m^{-2} s^{-1}
N.arcticum	5	50.2	73.6
	10	0.0	20.3
	17	0.0	16.5
C. mitis	5	61.3	86.3
	10	60.0	100.0
	17	-22.2	51.9
C. stellaris	5	27.9	84.0
	10	12.1	92.7
	17	20.0	47.0
C. rangiferina	5	0.0	65.7
	10	50.1	82.3
	17	63.1	57.1

6.1.3 Conclusions

From the results obtained in this study it is evident that each species has exhibited a significantly different response of thallus NP to air temperature at time of measurement, light intensity at time of measurement and temperature regime during storage (Tables 4.3.2 & 5.3.1). Furthermore, while some of the responses can be somewhat predicted by species morphology or habitat ecology, species traits such as light compensation of photosynthesis point, photoinhibition levels and the effect of temperature regime during storage do not seem to have exhibited patterns which can be attributed to species characteristics. Importantly *N. arcticum* thalli exhibited very similar responses to its abiotic environment to those that have been found in previous experiment on thalli from the same region (Sonesson *et al.*, 1992). Yet the reindeer lichens exhibited different responses, of NP to climatic factors, to the responses documented in thalli from North American populations of the same species (Adams, 1971; Ino, 1985; Kershaw *et al.*, 1983; Lechowicz, 1978; Lechowicz and Adams, 1974; Macfarlane *et al.*, 1983). The differences evident, in the responses of metabolic rate of thalli to climatic factors, between populations of the same species from different continents could have an ecological significance, where species react differently to variation of climatic factors

depending on where they are globally. This is evidence of a higher level of within species variation than has previously been exhibited in lichens, and suggests the importance of comparing the effect of climate on populations of lichen species from different geographic areas. An isoenzyme test (see Macfarlane *et al.*, 1983) looking for genetic differences between lichen populations, of the same species, from different geographical regions, may help describe the underlying causation of the variation in the response of lichens, of the same species from different regions, to their abiotic environment.

As the mean NP of each species significantly differed from each other, three-way ANOVA analysis in Chapter 5, and the effect of changing the abiotic environment of storage significantly interacted with species, it can be suggested that species from a similar climatic envelope are not similarly affected by changes in their abiotic environment. Even species of the same genus exhibited significantly different reactions to their abiotic environment. Although with comparison to results from Ino (1985) it is possible that lichen species with a foliose morphology exhibit as similar response of NP to temperature, there is no such evidence for fruiticose species. It can be concluded from this study lichen species, for the purpose of modelling, should not, ideally, be considered as a single organism, as it will most likely result in erroneous data.

A full analysis of how the environmental conditions of the individual niche habitats of each species, change through the year would substantially help identify environmental conditions that can used to predict how species will react to variations of their abiotic environment.

6.2 Seasonal variation of productivity in sub-Arctic lichens

6.2.1 Previous studies of sub-Arctic non-vascular productivity

Experiments on the effects of environmental conditions on the photosynthetic rate of lichens have often concluded that higher rates of NP are achieved during periods of warmer prevailing conditions (Benedict, 1990). Studies monitoring non-vascular productivity, during the Arctic peak vascular growth season, have found lichens do not significantly contribute to net ecosystem productivity, even though controlled temperature experiments suggest that the warmest months, i.e. peak vascular growth season, should be where maximal photosynthetic activity is achieved (Williams *et al.*, 2000). Conversely the introduction has provided examples of where raised air temperature has led directly to the loss of lichen biomass in an ecosystem (*see Chapter 1*). Many studies now conclude, however, that the productivity of lichens is more closely related to the length of wet active periods that a thallus is subjected to than the surrounding air temperature (Lange, 2002; Lange, 2003a; Lange, 2003b). Recent studies have successfully modelled the length of wet active time of lichens from the water potential of the surrounding environment; which is calculated from the air temperature and relative humidity (Jonsson *et al.*, 2008). However the annual net productivity of lichens cannot be accounted for solely by wet active time, and it has been shown in this report, and in similar studies, that the rates of NP of lichens, at optimal hydration, are significantly affected by thallus irradiance and air temperature (Cabrajic *et al.*, 2010; Kershaw *et al.*, 1983; Lange, 2002; Macfarlane *et al.*, 1983; Sonesson *et al.*, 1992). This would suggest that thallus irradiance and air temperature, during wet active periods, significantly affects the net annual productivity of lichens (Lange, 2003a; Lange, 2003b). Furthermore it can be suggested that lichens contribute to net ecosystem productivity at any time of the year, when they are hydrated and have sufficient PAR available for photosynthesis, and that the highest rate of contribution is achieved under optimum temperature regimes.

6.2.2 Climatic data from Abisko, Sweden

The mean surface moisture availability of the tundra-heath ecosystem during each month (data for 2007-8), in Abisko, and the air temperature at time of measurement, has been presented in Figure 6.2.1. If it is assumed that lichen thalli are capable of utilising environmental moisture in a similar fashion to how it has been measured by the moisture sensors (HMP45AC; Vaisala, Helsinki, Finland), then Figure 6.2.1 can be suggested to be a rough prediction of how wet active time of lichen species in the area changes through the year. The graph clearly illustrates that the length of time in which lichens can achieve metabolically active wet time is lowest during the mid-summer months July

and August; which is the period of peak vascular plant primary productivity, suggesting that the non-vascular contribution to NEP is minimal during this period (Campioli *et al.*, 2009; Williams *et al.*, 2000). Furthermore almost 50% of the time that lichen thalli were hydrated enough for metabolic activity to occur, in the month of July, the temperature was high enough to significantly depress the rate of thallus NP; in all species (Figures 6.1.1 and 6.2.1). The annual pattern of moisture availability is the same what-ever the moisture threshold is set as, however increasing the sensor resistance threshold results in a dramatic reduction of half hour periods in each month.

The results, from Chapter 4, have shown that summer adapted thalli (i.e. thalli kept at 15˚C with long-day irradiance), of the lichen species *N. arcticum*, achieve maximum NP at 5˚C, and thalli of *C. rangiferina*, at 10˚C. In summer adapted thalli of *C. stellaris* and *C. mitis* there was no significant difference between NP achieved at 10˚ and 5˚C. In *C. stellaris* and *C. mitis* it can be suggested that NP will occur at near maximal levels, irrespective of air temperature, throughout the snow-free months of the year, and will be closely related to thallus irradiance during periods of active wet time. From Chapter 5 it was shown that in all species studied, other than *C. mitis*, maximal NP was achieved under the 5˚C regime. If the length of time, during which hydration conditions are suitable for metabolic activity and that the air temperature is within the temperatures of the 5˚C climate regime, are plotted for each month of the year in which there is no snow it is evident that maximal NP of all species is mostly to occur *in situ* during May, June, September and October (Figure 6.2.2). This suggests that the lichen peak-growth season is much longer than the vascular peak-growth season and that in Arctic-mid-summer lichen primary productivity is significantly depressed.

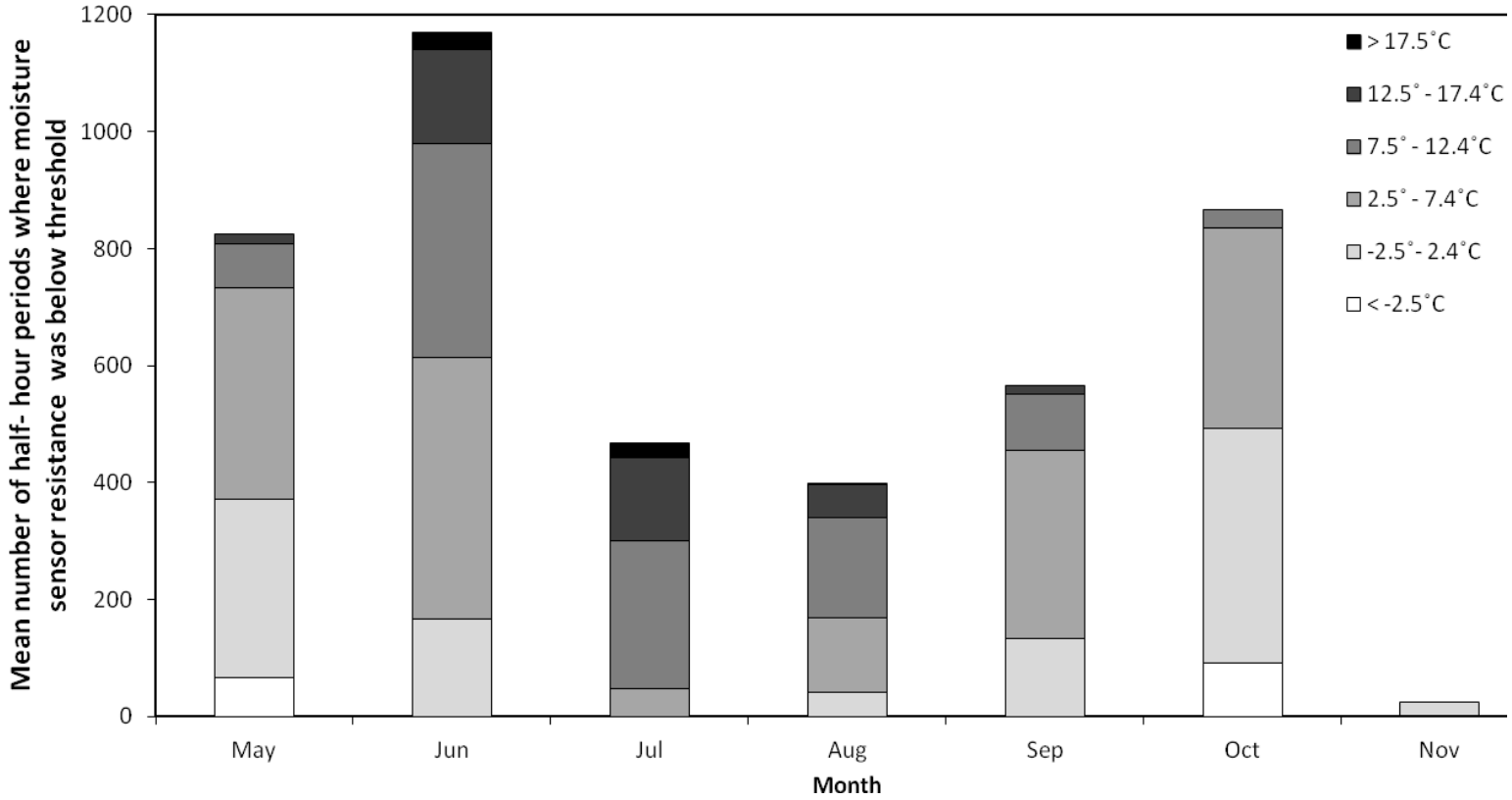

Figure 6.2.1: Histogram depicting mean number of half hour periods, in which moisture-sensor-resistance was below a threshold (threshold values were relative), in each month of the year. Periods are separated according to the air temperature at the time of measurement. Values were derived from climatological data from tundra-heath at Abisko, Sweden, during a two year period (2007-8) (Baxter, unpublished). The periods which occurred during night-time have been removed from the data; as lichens would not have sufficient PAR available for NP to occur. The proportional relationship between months stayed the same when the threshold was changed. Months in which the sensor was frozen have not been included.

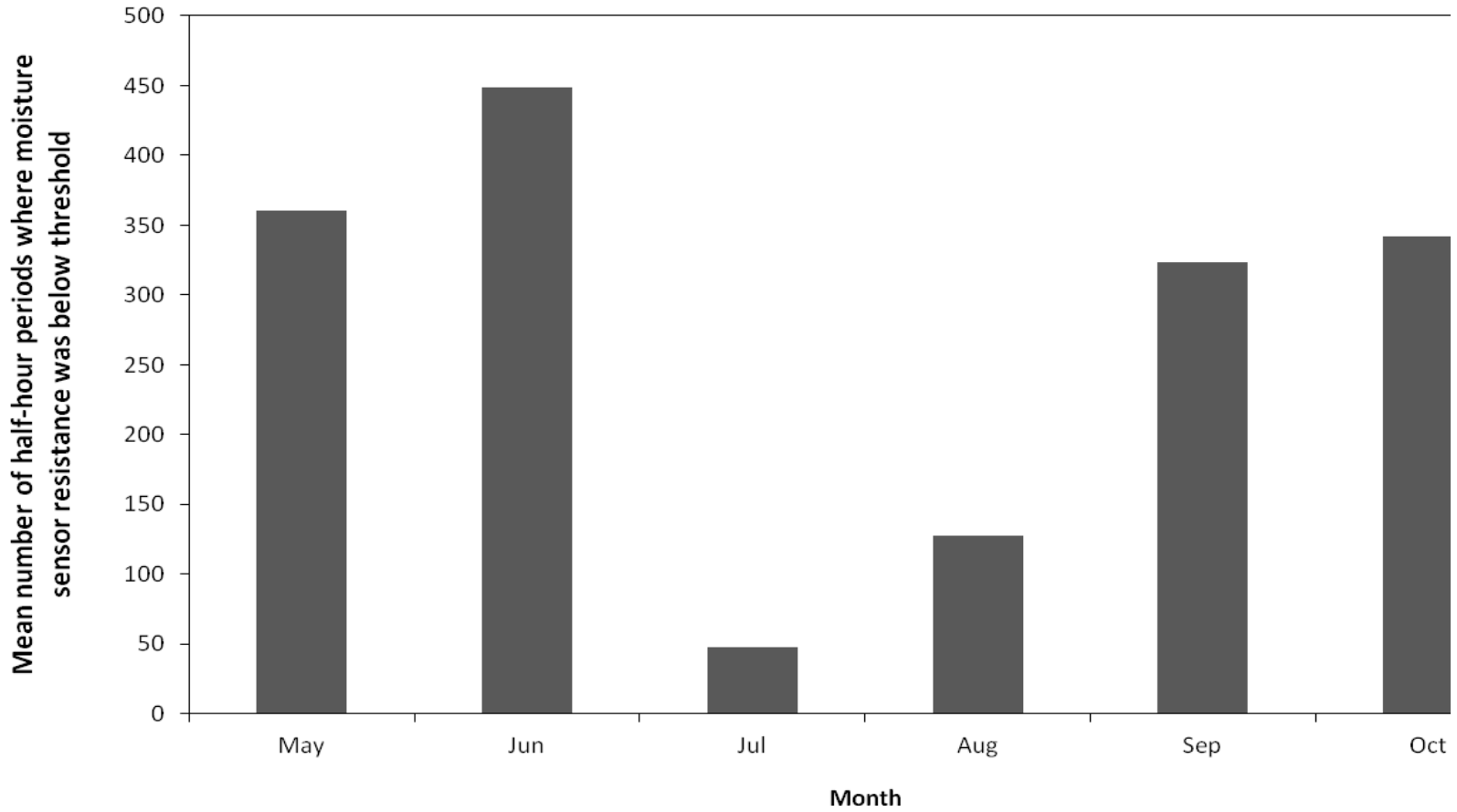

Figure 6.2.2: Histogram depicting mean number of half hour periods, in which moisture sensor resistance was below a threshold (threshold values were relative), at a temperature that would have allowed lichens to achieve maximal NP (2.5˚- 7.5˚C), in each month of the year. Values were derived from climatological data from Abisko, Sweden, during a two year period (2007-8) (Baxter, unpublished). The periods which occurred during night-time have been removed from the data; as lichens would not have sufficient PAR available for NP to occur. Data are only presented for the snow-free seasons.

6.2.3 Conclusions

The results obtained in this report suggest that much of Arctic-heath lichen productivity is achieved outside of the peak vascular plant growth period. The length of time through the year in which lichens may be capable of achieving NP, combined with the high biomass of the reindeer lichens within the ecosystem, suggests that these species may be providing an important, and unrecognised, contribution to NEP, in an ecosystem which is suggested to be an important global carbon sink (Ping *et al.*, 2008). The photosynthetic plasticity lichen species and previous evidence of seasonal photosynthetic adaption could, however, significantly affect the rate of thallus NP. Therefore a comprehensive test of the rate of carbon accumulation and how it changes through the year in thalli of the three *Cladina* species studied, in a similar fashion the studies by Sonesson *et al.* (1992) and Benedict (1990), in Fennoscandian tundra ecosystems, should be conducted to comprehensively prove the theory that lichens contribute to ecosystem productivity outside of the vascular peak growth season.

6.3 Effect of predicted future climate change on the ecology of the lichens species studied

The future climates of Arctic environments are predicted to generally be warmer, with a greater magnitude of warming occurring in average winter temperature than summer temperatures, and wetter, although Scandinavian summers are predicted to become drier (Hassol, 2004). This will most likely result in an increase in the length of tundra snow-free period and a longer peak vascular plant growth season; which has been shown to significantly increase vascular plant biomass (Walker *et al.*, 2006). Experimental warming studies, in the tundra biome, have provided a wide range of results in regard to lichen biomass: in some lichen biomass increased (Cabrajic, 2009), in some lichen biomass significantly decreased (Cornelissen *et al.*, 2001), and in some the response was species specific (Jagerbrand *et al.*, 2009). However studies of past climatic warming on lichens conclude that the diversity of cold-adapted lichens is significantly reduced by climatic warming events (Aptroot and van Herk, 2007; Hauck, 2009; van Herk *et al.*, 2002).

The conclusion that lichens adapted to colder environments, e.g. the lichen species studied, will exhibit a reduction in biomass due to climatic warming is supported by the results obtained in this report. In all species the rate of NP generally decreased as temperature was increased, this was especially true when the length of time that sample were exposed to a temperature increased in *C. stellaris*. Furthermore the light compensation point of photosynthesis, of all species, significantly increased with temperature (Table 4.3.3); suggesting that at higher temperatures higher light intensities are required for lichens to achieve NP. If predicted trends in global dimming are correct, or if climatic change results in increased vascular biomass, leading to increased shading of lichen thalli, then the detrimental effect of higher temperatures on lichen NP could be even greater than predicted in by models that do not factor in changes in light intensity (Jagerbrand *et al.*, 2009; Tommervik *et al.*, 2009; Walker *et al.*, 2006; Wild, 2009). However it is worth noting that an extension of the spring and autumn growth season, which have been shown in this report to be the months where moisture availability and temperatures are most suited to maximal productivity of sub-Arctic lichens (Figures 6.2.1 and 6.2.2), could be beneficial to lichen biomass in tundra-heath ecosystems; especially in areas of low vascular plant presence.

The lichens studied in this report comprise of a significant proportion of biomass in Arctic tundra-heath ecosystems (Arseneault *et al.*, 1997). Furthermore lichens in general comprise of a significant proportion of Arctic species diversity and photosynthetic biomass (Callaghan *et al.*, 2004b; Walker and Walker, 1996). Loss of cold-adapted lichen species within the Arctic could have some serious ecological implications; replacement of lichen biomass, with biomass of shrubby taxa, could result in an albedo-warming feedback loop, which could speed up the rate of Arctic warming and

intensify its predicted ecological consequences (Chapin *et al.*, 2005; Higuera *et al.*, 2008). Furthermore *Cladina* species lichens represent a significant proportion of winter forage of reindeer populations (Joly *et al.*, 2009; Roturier and Roue, 2009). Loss of forage species could lead to reductions in *Rangifer* populations wherever they occur; which would have a significant impact on the reindeer-herding nomadic peoples and speed up the expansion of shrubby taxa (Olofsson *et al.*, 2009; Roturier and Roue, 2009). Knowledge of how to redistribute and manage lichen species used as winter forage, could help to reduce the potentially devastating impact that loss of lichen species in the Arctic, due to future anthropogenic climate warming, could have upon nomadic populations within the Arctic biome.

7. Bibliography

Adamo, P. & Violante, P. (2000) Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science,* **16,** 229-256.

Adams, M.S. (1971) Temperature response of carbon dioxide exchange of *Cladonia rangiferina* from Wisconsin pine barrens, and comparison with an alpine population. *American Midland Naturalist,* **86,** 224-&.

Ahmadjian, V. (1993) *The lichen symbiosis.* John Wiley and Sons, Inc.; John Wiley and Sons Ltd.

Aptroot, A. & van Herk, C.M. (2007) Further evidence of the effects of global warming on lichens, particularly those with *Trentepohlia* phycobionts. *Environmental Pollution,* **146,** 293-298.

Arseneault, D., Villeneuve, N., Boismenu, C., Leblanc, Y. & Deshaye, J. (1997) Estimating lichen biomass and caribou grazing on the wintering grounds of northern Quebec: An application of fire history and Landsat data. *Journal of Applied Ecology,* **34,** 65-78.

Asplund, J. & Gauslaa, Y. (2008) Mollusc grazing limits growth and early development of the old forest lichen *Lobaria pulmonaria* in broadleaved deciduous forests. *Oecologia,* **155,** 93-99.

Asplund, J., Larsson, P., Vatne, S. & Gauslaa, Y. (2010) Gastropod grazing shapes the vertical distribution of epiphytic lichens in forest canopies. *Journal of Ecology,* **98,** 218-225.

Aubert, S., Juge, C., Boisson, A.M., Gout, E. & Bligny, R. (2007) Metabolic processes sustaining the reviviscence of lichen *Xanthoria elegans* (Link) in high mountain environments. *Planta,* **226,** 1287-1297.

Badger, M.R., Pfanz, H., Budel, B., Heber, U. & Lange, O.L. (1993) Evidence for the functioning of photosynthetic CO_2-concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta,* **191,** 57-70.

Baron, G. (1999) *Understanding Lichens.* The Richmond Publishing Company, Slough.

Bartak, M., Hajek, J., Vrablikova, H. & Dubova, J. (2004) High-light stress and photoprotection in *Umbilicaria antarctica* monitored by chlorophyll fluorescence Imaging and changes in zeaxanthin and glutathione. *Plant Biology,* **6,** 333-341.

Bartak, M., Vrablikova-Cempirkova, H., Stepigova, J., Hajek, J., Vaczi, P. & Vecerova, K. (2008) Duration of irradiation rather than quantity and frequency of high irradiance inhibits photosynthetic processes in the lichen *Lasallia pustulata*. *Photosynthetica,* **46,** 161-169.

Beckett, R.P. (1995) Some aspects of the water relations of lichens from habitats of contrasting water status studied using thermocouple psychrometry. *Annals of Botany,* **76,** 211-217.

Benedict, J.B. (1990) Experiments on lichen growth .1. Seasonal patterns and environmental controls. *Arctic and Alpine Research,* **22,** 244-254.

Biasi, C., Meyer, H., Rusalimova, O., Hammerle, R., Kaiser, C., Baranyi, C., Daims, H., Lashchinsky, N., Barsukov, P. & Richter, A. (2008) Initial effects of experimental warming on carbon exchange rates, plant growth and microbial dynamics of a lichen-rich dwarf shrub tundra in Siberia. *Plant and Soil,* **307,** 191-205.

Bjerke, J.W. (2009) Ice encapsulation protects rather than disturbs the freezing lichen. *Plant Biology,* **11,** 227-235.

Bjerke, J.W., Elvebakk, A., Dominguez, B. & Dahlback, A. (2005a) Seasonal trends in usnic acid concentrations of Arctic, alpine and Patagonian populations of the lichen *Flavocetraria nivalis*. *Phytochemistry,* **66,** 337-344.

Bjerke, J.W., Gwynn-Jones, D. & Callaghan, T.V. (2005b) Effects of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic-alpine lichens. *Environmental and Experimental Botany,* **53,** 139-149.

Breen, K. & Levesque, E. (2008) The influence of biological soil crusts on soil characteristics along a High Arctic glacier foreland, Nunavut, Canada. *Arctic Antarctic and Alpine Research,* **40,** 287-297.

Brock, T.D. (1975) Effect of water potential on photosynthesis in whole lichens and in their liberated algal components. *Planta,* **124,** 13-23.

Brodo, I.M., Sharnoff, S.D. & Sharnoff, S. (2001) *Lichens of North America.* Yale University Press, New Haven and London.

Brown, D. & Kershaw, K.A. (1984) Photosynthetic capacity changes in *Peltigera* .2. Contrasting season patterns of net photosynthesis in 2 populations of *Peltigera rufescens*. *New Phytologist,* **96,** 447-457.

Brown, D. & Kershaw, K.A. (1986) Seasonal-changes in the kinetic parameters of a photosynthetic fructose-1,6-bisphosphatase isolated from *Peltigera rufescens*. *Plant Physiology,* **82,** 457-461.

Cabrajic, A.V.J. (2009) *Modeling lichen performance in relation to climate- scaling from landscapes*. Umeå University, Umeå.

Cabrajic, A.V.J., Liden, M., Lundmark, T., Ottosson-Lofvenius, M. & Palmqvist, K. (2010) Modelling hydration and photosystem II activation in relation to in situ rain and humidity patterns: a tool to compare performance of rare and generalist epiphytic lichens. *Plant Cell and Environment,* **33,** 840-850.

Callaghan, T.V., Bjorn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., Oechel, W. & Shaver, G. (2004a) Past changes in arctic terrestrial ecosystems, climate and UV radiation. *Ambio,* **33,** 398-403.

Callaghan, T.V., Bjorn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., Oechel, W., Shaver, G., Elster, J., Henttonen, H., Laine, K., Taulavuori, K., Taulavuori, E. & Zockler, C. (2004b) Biodiversity, distributions and adaptations of arctic species in the context of environmental change. *Ambio,* **33,** 404-417.

Callaghan, T.V., Bjorn, L.O., Chernov, Y., Chapin, T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonasson, S., Matveyeva, N., Panikov, N., Oechel, W., Shaver, G., Elster, J., Jonsdottir, I.S., Laine, K., Taulavuori, K., Taulavuori, E. & Zockler, C. (2004c) Responses to projected changes in climate and UV-B at the species level. *Ambio,* **33,** 418-435.

Campioli, M., Samson, R., Michelsen, A., Jonasson, S., Baxter, R. & Lemeur, R. (2009) Nonvascular contribution to ecosystem NPP in a subarctic heath during early and late growing season. *Plant Ecology,* **202,** 41-53.

Chapin, F.S., Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J. & Laundre, J.A. (1995) Responses of Arctic tundra to experimental and observed changes in climate. *Ecology,* **76,** 694-711.

Chapin, F.S., Sturm, M., Serreze, M.C., McFadden, J.P., Key, J.R., Lloyd, A.H., McGuire, A.D., Rupp, T.S., Lynch, A.H., Schimel, J.P., Beringer, J., Chapman, W.L., Epstein, H.E., Euskirchen, E.S., Hinzman, L.D., Jia, G., Ping, C.L., Tape, K.D., Thompson, C.D.C., Walker, D.A. & Welker, J.M. (2005) Role of land-surface changes in Arctic summer warming. *Science,* **310,** 657-660.

Conti, M.E. & Cecchetti, G. (2001) Biological monitoring: lichens as bioindicators of air pollution assessment - a review. *Environmental Pollution,* **114,** 471-492.

Cornelissen, J.H.C., Callaghan, T.V., Alatalo, J.M., Michelsen, A., Graglia, E., Hartley, A.E., Hik, D.S., Hobbie, S.E., Press, M.C., Robinson, C.H., Henry, G.H.R., Shaver, G.R., Phoenix, G.K., Jones, D.G., Jonasson, S., Chapin, F.S., Molau, U., Neill, C., Lee, J.A., Melillo, J.M., Sveinbjornsson, B. & Aerts, R. (2001) Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass? *Journal of Ecology,* **89,** 984-994.

Cornelissen, J.H.C., Lang, S.I., Soudzilovskaia, N.A. & During, H.J. (2007) Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany,* **99,** 987-1001.

Coulson, S.J., Hodkinson, I.D., Webb, N.R., Block, W., Bale, J.S., Strathdee, A.T., Worland, M.R. & Wooley, C. (1996) Effects of experimental temperature elevation on high-arctic soil microarthropod populations. *Polar Biology,* **16,** 147-153.

Cowan, I.R., Lange, O.L. & Green, T.G.A. (1992) Carbon-dioxide exchange in lichens - determination of transport and carboxylation characteristics. *Planta,* **187,** 282-294.

Coxson, D.S., Brown, D. & Kershaw, K.A. (1983) The interaction between CO_2 diffusion and the degree of thallus hydration in lichens - some further comments. *New Phytologist,* **93,** 247-260.

Coxson, D.S. & Wilson, J.A. (2004) Carbon gain in *Cladina mitis* from mixed feather moss mats in a sub-alpine spruce-fir forest: The role of soil respiratory carbon dioxide release. *Symbiosis,* **37,** 307-321.

Dahlman, L. & Palmqvist, K. (2003) Growth in two foliose tripartite lichens, *Nephroma arcticum* and *Peltigera aphthosa*: empirical modelling of external vs internal factors. *Functional Ecology,* **17,** 821-831.

Davey, M.C., Pickup, J. & Block, W. (1992) Temperature-variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarctic Science,* **4,** 383-388.

Demmig-Adams, B., Maguas, C., Adams, W.W., Meyer, A., Kilian, E. & Lange, O.L. (1990) Effect of high light on the efficieny of photochemical energy- conversion in a variety of lichen species with green and blue-green phycobionts. *Planta,* **180,** 400-409.

Dokuchaev, N.E. (2009) On lichen-based feeding of the Red-backed vole (*Clethrionomys rutilus*). *Zoologichesky Zhurnal,* **88,** 1013-1016.

Eliseev, A.V., Arzhanov, M.M., Demchenko, P.F. & Mokhov, II (2009) Changes in climatic characteristics of Northern Hemisphere extratropical land in the 21st century: Assessments with the IAP RAS climate model. *Izvestiya Atmospheric and Oceanic Physics,* **45,** 271-283.

Gaio-Oliveira, G., Moen, J., Danell, O. & Palmqvist, K. (2006) Effect of simulated reindeer grazing on the re-growth capacity of mat-forming lichens. *Basic and Applied Ecology,* **7,** 109-121.

Gasulla, F., de Nova, P.G., Esteban-Carrasco, A., Zapata, J.M., Barreno, E. & Guera, A. (2009) Dehydration rate and time of desiccation affect recovery of the lichenic algae *Trebouxia erici*: alternative and classical protective mechanisms. *Planta,* **231,** 195-208.

Gauslaa, Y. (2006) Trade-off between reproduction and growth in the foliose old forest lichen *Lobaria pulmonaria*. *Basic and Applied Ecology,* **7,** 455-460.

Gauslaa, Y. & Solhaug, K.A. (1998) The significance of thallus size for the water economy of the cyanobacterial old-forest lichen *Degelia plumbea*. *Oecologia,* **116,** 76-84.

Gauslaa, Y. & Solhaug, K.A. (1999) High-light damage in air-dry thalli of the old forest lichen *Lobaria pulmonaria* - interactions of irradiance, exposure duration and high temperature. *Journal of Experimental Botany,* **50,** 697-705.

Genty, B., Briantais, J.M. & Baker, N.R. (1989) The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica Et Biophysica Acta,* **990,** 87-92.

Green, T.G.A., Schroeter, B., Kappen, L., Seppelt, R.D. & Maseyk, K. (1998) An assessment of the relationship between chlorophyll a fluorescence and CO_2 gas exchange from field measurements on a moss and lichen. *Planta,* **206,** 611-618.

Grellmann, D. (2002) Plant responses to fertilization and exclusion of grazers on an arctic tundra heath. *Oikos,* **98,** 190-204.

Groulx, M. & Lechowicz, M.J. (1987) Net photosynthesis recovery in sub-arctic lichens with contrasting water relations. *Oecologia,* **71,** 360-368.

Gunderson, C.A., O'Hara, K.H., Campion, C.M., Walker, A.V. & Edwards, N.T. (2010) Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate. *Global Change Biology,* **16,** 2272-2286.

Hajek, J., Bartak, M. & Gloser, J. (2001) Effects of thallus temperature and hydration on photosynthetic parameters of *Cetraria islandica* from contrasting habitats. *Photosynthetica,* **39,** 427-435.

Hansatech (2006) *Operations Manual: Oxygraph System.* Hansatech Instruments Ltd, King's Lynn.
Harrisson, P.M., Walton, D.W.H. & Rothery, P. (1986) The effects of temperature and moisture on dark respiration in the foliose lichen *Umbilicaria antarctica*. *New Phytologist,* **103,** 443-455.
Hartard, B., Cuntz, M., Maguas, C. & Lakatos, M. (2009) Water isotopes in desiccating lichens. *Planta,* **231,** 179-193.
Hassol, S.J. (2004) *Impacts of a warming Arctic: Arctic climate impact assessment.* Cambridge University Press, New York.
Hauck, M. (2009) Global warming and alternative causes of decline in arctic-alpine and boreal-montane lichens in North-Western Central Europe. *Global Change Biology,* **15,** 2653-2661.
Hawksworth, D.L., Kirk, P.M., Sutton, B.C. & Pegler, D.N. (1995) *Ainsworth and Bisby's dictionary of the fungi (8th edn).* CAB International, Wallingford.
Heatwole, H. (1966) Moisture exchange between atmosphere and some lichens of genus *Cladonia*. *Mycologia,* **58,** 148-&.
Heber, U. (2008) Photoprotection of green plants: a mechanism of ultra-fast thermal energy dissipation in desiccated lichens. *Planta,* **228,** 641-650.
Heber, U., Bilger, W., Turk, R. & Lange, O.L. (2010) Photoprotection of reaction centres in photosynthetic organisms: mechanisms of thermal energy dissipation in desiccated thalli of the lichen *Lobaria pulmonaria*. *New Phytologist,* **185,** 459-470.
Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lucking, R., Lumbsch, H.T., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Koljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schussler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J. & Zhang, N. (2007) A higher-level phylogenetic classification of the Fungi. *Mycological Research,* **111,** 509-547.
Higuera, P.E., Brubaker, L.B., Anderson, P.M., Brown, T.A., Kennedy, A.T. & Hu, F.S. (2008) Frequent fires in ancient shrub tundra: implications of paleorecords for arctic environmental change. *PLoS One,* **3,** e0001744.
Hodkinson, I.D., Coulson, S.J. & Webb, N.R. (2004) Invertebrate community assembly along proglacial chronosequences in the high Arctic. *Journal of Animal Ecology,* **73,** 556-568.
Hodkinson, I.D., Coulson, S.J., Webb, N.R. & Block, W. (1996) Can high Arctic soil microarthropods survive elevated summer temperatures? *Functional Ecology,* **10,** 314-321.
Hollister, R.D., Webber, P.J. & Tweedie, C.E. (2005) The response of Alaskan arctic tundra to experimental warming: differences between short- and long-term responses. *Global Change Biology,* **11,** 525-536.
Holt, E.A., McCune, B. & Neitlich, P. (2008) Grazing and fire impacts on macrolichen communities of the Seward Peninsula, Alaska, USA. *Bryologist,* **111,** 68-83.
Honegger, R. (1998) The Lichen symbiosis - What is so spectacular about it? *Lichenologist,* **30,** 193-212.
Huneck, S. (1999) The Significance of Lichens and Their Metabolites. *Naturwissenschaften,* **86,** 559-570.
Ihda, T.A., Nakano, T., Yoshimura, I. & Iwatsuki, Z. (1993) Phycobionts isolated from Japanese species of Anzia (Lichenes). *Archiv Fur Protistenkunde,* **143,** 163-172.
Ino, Y. (1985) Comparative study of the effects of temperature on the net photosynthesis respiration in lichens from the Antarctic and subalpine zones in Japan. *Botanical Magazine-Tokyo,* **98,** 41-53.

Jagerbrand, A.K., Alatalo, J.M., Chrimes, D. & Molau, U. (2009) Plant community responses to 5 years of simulated climate change in meadow and heath ecosystems at a subarctic-alpine site. *Oecologia,* **161,** 601-610.

Joly, K., Jandt, R.R. & Klein, D.R. (2009) Decrease of lichens in Arctic ecosystems: the role of wildfire, caribou, reindeer, competition and climate in north-western Alaska. *Polar Research,* **28,** 433-442.

Jonsson, A.V., Moen, J. & Palmqvist, K. (2008) Predicting lichen hydration using biophysical models. *Oecologia,* **156,** 259-273.

Kaplan, J.O., Bigelow, N.H., Prentice, I.C., Harrison, S.P., Bartlein, P.J., Christensen, T.R., Cramer, W., Matveyeva, N.V., McGuire, A.D., Murray, D.F., Razzhivin, V.Y., Smith, B., Walker, D.A., Anderson, P.M., Andreev, A.A., Brubaker, L.B., Edwards, M.E. & Lozhkin, A.V. (2003) Climate change and Arctic ecosystems: 2. Modeling, paleodata-model comparisons, and future projections. *Journal of Geophysical Research-Atmospheres,* **108**.

Kappen, L. (2000) Some aspects of the great success of lichens in Antarctica. *Antarctic Science,* **12,** 314-324.

Kappen, L. & Breuer, M. (1991) Ecological and physiological investigations in continental Antarctic cryptogams .2. Moisture relations and photosynthesis of lichens near Casey station, Wilkes land. *Antarctic Science,* **3,** 273-278.

Kappen, L., Schroeter, B., Green, T.G.A. & Seppelt, R.D. (1998) Microclimatic conditions, meltwater moistening, and the distributional pattern of *Buellia frigida* on rock in a southern continental Antarctic habitat. *Polar Biology,* **19,** 101-106.

Kappen, L., Sommerkorn, M. & Schroeter, B. (1995) Carbon acquisition and water relations of lichens in polar regions - Potentials and limitations. *Lichenologist,* **27,** 531-545.

Kershaw, K.A. (1977) Physiological environmental interactions in lichens .2. Pattern of net photosynthetic acclimation in *Peltigera canina* (L) Willd var *praetextata* (Floerke in Somm) Hue, and *Peltigera polydactyla* (Neck) Hoffm. *New Phytologist,* **79,** 377-390.

Kershaw, K.A. (1985) Physiological ecology of lichens. *Cambridge Studies in Ecology*, pp. 293. Cambridge University Press, Cambridge.

Kershaw, K.A., Macfarlane, J.D., Webber, M.R. & Fovargue, A. (1983) Phenotypic differences in the seasonal pattern of net photosynthesis in *Cladonia stellaris*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **61,** 2169-2180.

Knops, J.M.H., Nash, T.H. & Schlesinger, W.H. (1996) The influence of epiphytic lichens on the nutrient cycling of a blue oak woodland. (ed N. H. Pillsbury), pp. 75-82. Us Dept Agr, Forest Serv Pacific Sw Forest & Range Exptl Stn, San Luis Obispo, Ca.

Korner, C. (1999) *Alpine Plant Life.* Springer-Verlag, Berlin.

Kosugi, M., Arita, M., Shizuma, R., Moriyama, Y., Kashino, Y., Koike, H. & Satoh, K. (2009) Responses to Desiccation Stress in Lichens are Different from Those in Their Photobionts. *Plant and Cell Physiology,* **50,** 879-888.

Kranner, I., Beckett, R., Hochman, A. & Nash, T.H. (2008) Desiccation-tolerance in lichens: a review. *Bryologist,* **111,** 576-593.

Krzewicka, B. & Smykla, J. (2004) The lichen genus *Umbilicaria* from the neighbourhood of Admiralty Bay (King George Island, maritime Antarctic), with a proposed new key to all Antarctic taxa. *Polar Biology,* **28,** 15-25.

Lang, S.I., Cornelissen, J.H.C., Holzer, A., ter Braak, C.J.F., Ahrens, M., Callaghan, T.V. & Aerts, R. (2009) Determinants of cryptogam composition and diversity in *Sphagnum*-dominated peatlands: the importance of temporal, spatial and functional scales. *Journal of Ecology,* **97,** 299-310.

Lange, O.L. (2002) Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: Long-term field monitoring of CO_2 exchange and its physiological interpretation - I. Dependence of photosynthesis on water content, light, temperature, and CO_2 concentration from laboratory measurements. *Flora,* **197,** 233-249.

Lange, O.L. (2003a) Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO_2 exchange and its physiological interpretation - III. Diel, seasonal, and annual carbon budgets. *Flora,* **198,** 277-292.

Lange, O.L. (2003b) Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO_2 exchange and its physiological interpretation II. Diel and seasonal patterns of net photosynthesis and respiration. *Flora,* **198,** 55-70.

Lange, O.L. & Green, T.G.A. (1996) High thallus water content severely limits photosynthetic carbon gain of central European epilithic lichens under natural conditions. *Oecologia,* **108,** 13-20.

Lange, O.L., Green, T.G.A. & Heber, U. (2001) Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance? *Journal of Experimental Botany,* **52,** 2033-2042.

Lange, O.L., Green, T.G.A., Reichenberger, H. & Meyer, A. (1996) Photosynthetic depression at high thallus water contents in lichens: Concurrent use of gas exchange and fluorescence techniques with a cyanobacterial and a green algal *Peltigera* species. *Botanica Acta,* **109,** 43-50.

Lange, O.L., Green, T.G.A. & Ziegler, H. (1988) Water status related photosynthesis and carbon isotope discrimination in species of the lichen genus *Pseudocyphellaria* with green or blue-green photobionts and in Photosymbiodemes. *Oecologia,* **75,** 494-501.

Lange, O.L., Hahn, S.C., Meyer, A. & Tenhunen, J.D. (1998) Upland tundra in the foothills of the Brooks Range, Alaska, USA: Lichen long-term photosynthetic CO_2 uptake end net carbon gain. *Arctic and Alpine Research,* **30,** 252-261.

Lange, O.L., Kilian, E. & Ziegler, H. (1986) Water-vapour uptake and photosynthesis of lichens - performance differences in species with green and blue-green algae as phycobionts. *Oecologia,* **71,** 104-110.

Lange, O.L., Reichenberger, H. & Walz, H. (1997) Continuous monitoring of CO_2 exchange of lichens in the field: Short-term enclosure with an automatically operating cuvette. *Lichenologist,* **29,** 259-274.

Larson, D.W. (1982) Environmental-stress and *Umbilicaria* lichens - the effect of subzero temperature pretreatments. *Oecologia,* **55,** 268-278.

Larson, D.W. (1989) The impact of 10 years at -20 degrees C on gas-exchange in 5 lichen species. *Oecologia,* **78,** 87-92.

Larsson, P., Vecerova, K., Cempirkova, H., Solhaug, K.A. & Gauslaa, Y. (2009) Does UV-B influence biomass growth in lichens deficient in sun-screening pigments? *Environmental and Experimental Botany,* **67,** 215-221.

Lattman, H., Milberg, P., Palmer, M.W. & Mattsson, J.E. (2009) Changes in the distributions of epiphytic lichens in southern Sweden using a new statistical method. *Nordic Journal of Botany,* **27,** 413-418.

Laurent, J.M., Francois, L., Bar-Hen, A., Bel, L. & Cheddadi, R. (2004) European bioclimatic affinity groups: Data-model comparisons. pp. 28-40. Elsevier Science Bv, Florence, ITALY.

Leary, D.J. & Petchey, O.L. (2009) Testing a biological mechanism of the insurance hypothesis in experimental aquatic communities. *Journal of Animal Ecology,* **78,** 1143-1151.

Lechowicz, M.J. (1978) Carbon dioxide exchange in *Cladina* lichens from sub-arctic and temperate habitats. *Oecologia,* **32,** 225-237.

Lechowicz, M.J. (1982) Ecological trends in lichen photosysthesis. *Oecologia,* **53,** 330-336.

Lechowicz, M.J. & Adams, M.S. (1974) Ecology of *Cladonia* lichens .2. Comparative physiological ecology of *C. mitis*, *C. rangeferina* and *C. uncialis*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **52,** 411-422.

Lepetz, V., Massot, M., Schmeller, D.S. & Clobert, J. (2009) Biodiversity monitoring: some proposals to adequately study species' responses to climate change. *Biodiversity and Conservation,* **18,** 3185-3203.

Liden, M., Cabrajic, A.V.J., Ottosson-Lofvenius, M., Palmqvist, K. & Lundmark, T. (2010) Species-specific activation time-lags can explain habitat restrictions in hydrophilic lichens. *Plant Cell and Environment,* **33,** 851-862.

Llano, G. (1948) Economic uses of lichens. *Economic Botany,* **2,** 15-45.

Lutzoni, F., Pagel, M. & Reeb, V. (2001) Major fungal lineages are derived from lichen symbiotic ancestors. *Nature,* **411,** 937-940.

Macfarlane, J.D., Kershaw, K.A. & Webber, M.R. (1983) Physiological environmental interactions in lichens .17. Phenotypic differences in the seasonal pattern of net photosynthesis in *Cladonia rangiferina*. *New Phytologist,* **94,** 217-233.

MacKenzie, T.D.B., Krol, M., Huner, N.P.A. & Campbell, D.A. (2002) Seasonal changes in chlorophyll fluorescence quenching and the induction and capacity of the photoprotective xanthophyll cycle in *Lobaria pulmonaria*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **80,** 255-261.

Manrique, E., Balaguer, L., Barnes, J. & Davison, A.W. (1993) Photoinhibition studies in lichens using chlorophyll fluorescence analysis. *Bryologist,* **96,** 443-449.

Matlack, G.R. (1993) Microenvironment variation within and among forest edge sites in the eastern United States. *Biological Conservation,* **66,** 185-194.

Meier, F.A., Scherrer, S. & Honegger, R. (2002) Faecal pellets of lichenivorous mites contain viable cells of the lichen-forming ascomycete *Xanthoria parietina* and its green algal photobiont, *Trebouxia arboricola*. *Biological Journal of the Linnean Society,* **76,** 259-268.

Minibayeva, F. & Beckett, R.P. (2001) High rates of extracellular superoxide production in bryophytes and lichens, and an oxidative burst in response to rehydration following desiccation. *New Phytologist,* **152,** 333-341.

Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R. & Laundre, J.A. (1991) Effects of temperature and substrate quality on element mineralisation in 6 Arctic soils. *Ecology,* **72,** 242-253.

Nash, T.H. (1996) Photosynthesis, respiration, productivity and growth. *Lichen Biology* (ed T. H. Nash). Cambridge University Press, Cambridge.

Nybakken, L., Johansson, O. & Palmqvist, K. (2009) Defensive compound concentration in boreal lichens in response to simulated nitrogen deposition. *Global Change Biology,* **15,** 2247-2260.

Oliver, M.J., Tuba, Z. & Mishler, B.D. (2000) The evolution of vegetative desiccation tolerance in land plants. *Plant Ecology,* **151,** 85-100.

Olofsson, J., Oksanen, L., Callaghan, T., Hulme, P.E., Oksanen, T. & Suominen, O. (2009) Herbivores inhibit climate-driven shrub expansion on the tundra. *Global Change Biology,* **15,** 2681-2693.

Overpeck, J., Hughen, K., Hardy, D., Bradley, R., Case, R., Douglas, M., Finney, B., Gajewski, K., Jacoby, G., Jennings, A., Lamoureux, S., Lasca, A., MacDonald, G., Moore, J., Retelle, M., Smith, S., Wolfe, A. & Zielinski, G. (1997) Arctic environmental change of the last four centuries. *Science,* **278,** 1251-1256.

Oyarzun, C.E., Godoy, R., De Schrijver, A., Staelens, J. & Lust, N. (2004) Water chemistry and nutrient budgets in an undisturbed evergreen rainforest of southern chile. *Biogeochemistry,* **71,** 107-123.

Pallant, J. (2007) *SPSS Survival Manual.* Open University Press - McGraw-Hill Education, Maidenhead.

Palmqvist, K. & Dahlman, L. (2006) Responses of the green algal foliose lichen Platismatia glauca to increased nitrogen supply. *New Phytologist,* **171,** 343-356.

Palmqvist, K. & Sundberg, B. (2000) Light use efficiency of dry matter gain in five macro-lichens: relative impact of microclimate conditions and species-specific traits. *Plant Cell and Environment,* **23,** 1-14.

Pannewitz, S., Schlensog, M., Green, T.G.A., Sancho, L.G. & Schroeter, B. (2003) Are lichens active under snow in continental Antarctica? *Oecologia,* **135,** 30-38.

Petchey, O.L., McPhearson, P.T., Casey, T.M. & Morin, P.J. (1999) Environmental warming alters food-web structure and ecosystem function. *Nature,* **402,** 69-72.

Ping, C.L., Michaelson, G.J., Jorgenson, M.T., Kimble, J.M., Epstein, H., Romanovsky, V.E. & Walker, D.A. (2008) High stocks of soil organic carbon in the North American Arctic region. *Nature Geoscience,* **1,** 615-619.

Reiter, R., Hoftberger, M., Green, T.G.A. & Turk, R. (2008) Photosynthesis of lichens from lichen-dominated communities in the alpine/nival belt of the Alps - II: Laboratory and field measurements of CO2 exchange and water relations. *Flora,* **203,** 34-46.

Ringius, G.S. & Sims, R.A. (1997) Indicator plant species in Canadian Forests. Natural Resources Canada, Canadian Forest Service, Ottawa, ON.

Rogers, R.W. (1971) Distribution of lichen *Chondropsis semiviridis* in relation to its heat and drought resistance. *New Phytologist,* **70,** 1069-&.

Roturier, S. & Bergsten, U. (2009) Establishment of *Cladonia stellaris* after artificial dispersal in an unfenced forest in northern Sweden. *Rangifer,* **29,** 39-49.

Roturier, S. & Roue, M. (2009) Of forest, snow and lichen: Sami reindeer herders' knowledge of winter pastures in northern Sweden. *Forest Ecology and Management,* **258,** 1960-1967.

Sancho, L.G., De La Torre, R. & Pintado, A. (2008) Lichens, new and promising material from experiments in astrobiology. *Fungal Biology Reviews,* **22,** 103-109.

Schipperges, B. (1992) Patterns of CO_2 gas-exchange and thallus water-content in Arctic lichens along a ridge profile near Ny-Alesund, Svalbard. *Polar Research,* **11,** 47-68.

Schipperges, B. & Gehrke, C. (1996) Photosynthetic characteristics of Subarctic mosses and lichens *Ecological Bulletins,* **45,** 121-126.

Schroeter, B., Kappen, L., Green, T.G.A. & Seppelt, R.D. (1996) Lichens and the Antarctic environment: Effects of temperature and water availability on photosynthesis. (eds W. B. Lyons, C. HowardWilliams & I. Hawes), pp. 103-117. A a Balkema Publishers, Christchurch, New Zealand.

Schroeter, B. & Scheidegger, C. (1995) Water relations in lichens at subzero temperatures - structural-changes and carbon-dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica. *New Phytologist,* **131,** 273-285.

Seaward, M.R.D. (1976) Performance of *Leconora muralis* in an urban environment. *Lichenology: progress and problems* (eds D. H. Brown, D. L. Hawksworth & R. H. Bailey), pp. 323-358. Academic Press, London.

Serreze, M.C., Walsh, J.E., Chapin, F.S., Osterkamp, T., Dyurgerov, M., Romanovsky, V., Oechel, W.C., Morison, J., Zhang, T. & Barry, R.G. (2000) Observational evidence of recent change in the northern high-latitude environment. *Climatic Change,* **46,** 159-207.

Sharp, P.J.H. (1983) Responses of photosynthesis and dark respiration to temperature. *Annals of Botany,* **52,** 325-343.

Shaver, G.R., Bret-Harte, S.M., Jones, M.H., Johnstone, J., Gough, L., Laundre, J. & Chapin, F.S. (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology,* **82,** 3163-3181.

Shaver, G.R. & Chapin, F.S. (1991) Production - biomass relationships and element cycling in contrasting Arctic vegetation types. *Ecological Monographs,* **61,** 1-31.

Shimizu, A. & Kubo, T. (2009) Relationship between thallus size and apothecium density in two alpine umbilicate lichens. *Lichenologist,* **41,** 285-297.

Showman, R.E. (1979) Cold injury to lichens in Southern Ohio. *Bryologist,* **82,** 620-621.

Sigfridsson, B. (1980) Some effects of humidity on the light reaction of photosynthesis in the lichens *Cladonia impexa* and *Collema flaccidium*. *Physiologia Plantarum,* **49,** 320-326.

Sipman, H.J.M. & Aptroot, A. (2001) Where are the missing lichens? *Mycological Research,* **105,** 1433-1439.

Skre, O., Baxter, R., Crawford, R.M.M., Callaghan, T.V. & Fedorkov, A. (2002) How will the tundra-taiga interface respond to climate change? *Ambio***,** 37-46.

Solhaug, K.A., Gauslaa, Y., Nybakken, L. & Bilger, W. (2003) UV-induction of sun-screening pigments in lichens. *New Phytologist,* **158,** 91-100.

Sonesson, M. (1989) Water, light and temperature relations of the epiphytic lichens *Parmelia olivacea* and *Parmeliopsis ambigua* in Northern Swedish Lapland. *Oikos,* **56,** 402-415.

Sonesson, M., Schipperges, B. & Carlsson, B.A. (1992) Seasonal patterns of photosynthesis in alpine and sub-alpine populations of the lichen *Nephroma arcticum*. *Oikos,* **65,** 3-12.

Sundberg, B., Ekblad, A., Nasholm, T. & Palmqvist, K. (1999) Lichen respiration in relation to active time, temperature, nitrogen and ergosterol concentrations. *Functional Ecology,* **13,** 119-125.

Svensson, B.M. & Callaghan, T.V. (1988) Small-scale vegetation pattern related to the growth of *Lycopodium annotinum* and variations in its micro-environment. *Vegetatio,* **76,** 167-177.

Tearle, P.V. (1987) Cryptogamic carbohydrate release and microbial response during Spring freeze thaw cycles in Antarctic fellfield fines. *Soil Biology & Biochemistry,* **19,** 381-390.

Tegler, B. & Kershaw, K.A. (1980) Studies on lichen dominated systems 23. The control of seasonal rates of net photosynthesis by moisture, light and temperature in *Cladonia rangiferina*. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **58,** 1851-1858.

Tenhunen, J.D., Lange, O.L., Gebel, J., Beyschlag, W. & Weber, J.A. (1984) Changes in photosynthetic capacity, carboxylation efficiency, and CO_2 compensation point associated with midday stomatal closure and midday depression of net CO_2 exchange of leaves of *Quercus suber*. *Planta,* **162,** 193-203.

Tommervik, H., Johansen, B., Riseth, J.A., Karlsen, S.R., Solberg, B. & Hogda, K.A. (2009) Above ground biomass changes in the mountain birch forests and mountain heaths of Finnmarksvidda, northern Norway, in the period 1957-2006. *Forest Ecology and Management,* **257,** 244-257.

Tretiach, M. & Brown, D.H. (1995) Morphological and physiological differences between epilithic and epiphytic populations of the lichen *Parmelia pastillifera*. *Annals of Botany,* **75,** 627-632.

Uchida, M., Nakatsubo, T., Kanda, H. & Koizumi, H. (2006) Estimation of the annual primary production of the lichen *Cetrariella delisei* in a glacier foreland in the High Arctic, Ny-Alesund, Svalbard. *Polar Research,* **25,** 39-49.

Van Cleve, K., Oechel, W.C. & Hom, J.L. (1990) Response of black spruce *Picea mariana* ecosystems to soil temperature modification in interior Alaska USA. *Canadian Journal of Forest Research,* **20,** 1530-1535.

van Herk, C.M., Aptroot, A. & van Dobben, H.F. (2002) Long-tern monitoring in the Netherlands suggests that lichens respond to global warming. *Lichenologist,* **34,** 141-154.

Vitousek, P.M. (1994) Potential Nitrogen fixation during primary succession in hawaii volcanos national park. *Biotropica,* **26,** 234-240.

Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen limitation on land and in the sea - how can it occur. *Biogeochemistry,* **13,** 87-115.

Vrablikova, H., McEvoy, M., Solhaug, K.A., Bartak, M. & Gauslaa, Y. (2006) Annual variation in photo acclimation and photoprotection of the photobiont in the foliose lichen *Xanthoria parietina*. *Journal of Photochemistry and Photobiology B-Biology,* **83,** 151-162.

Walker, D.A. & Walker, M.D. (1996) Terrain and vegetation of the Imnavait Creek watershed. *Landscape Function and Disturbance in Arctic Tundra, Ecological Studies 120* (eds J. F. Reynolds & J. D. Ten-hunen), pp. 71-108. Springer-Verlag, Heidelburg, New York.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, M.S., Calef, M.P., Callaghan, T.V., Carroll, A.B., Epstein, H.E., Jonsdottir, I.S., Klein, J.A., Magnusson, B., Molau, U., Oberbauer, S.F., Rewa, S.P., Robinson, C.H., Shaver, G.R., Suding, K.N., Thompson, C.C., Tolvanen, A., Totland, O., Turner, P.L., Tweedie, C.E., Webber, P.J. & Wookey, P.A. (2006) Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences of the United States of America,* **103,** 1342-1346.

Wild, M. (2009) Global dimming and brightening: A review. *Journal of Geophysical Research-Atmospheres,* **114,** 31.

Williams, M., Eugster, W., Rastetter, E.B., McFadden, J.P. & Chapin, F.S. (2000) The controls on net ecosystem productivity along an Arctic transect: a model comparison with flux measurements. *Global Change Biology,* **6,** 116-126.

Zotz, G., Schweikert, A., Jetz, W. & Westerman, H. (2000) Water relations and carbon gain are closely related to cushion size in the moss *Grimmia pulvinata*. *New Phytologist,* **148,** 59-67.

Appendix 1

Figure 1: Mountain birch-forest in Abisko, Sweden. Favoured natural habitat of *N. arcticum* and possible habitat of *C. rangiferina* (see Figure 2.1.1). Figure depicts a similar environment from which *N. arcticum* samples were obtained. Image property of Dr. Robert Baxter.

Figure 2: Arctic tundra-heath in Abisko, Sweden. Whitish flora are *C. stellaris* thalli. Samples of *Cladina spp.* were collected from a similar environment. Image property of Dr. Robert Baxter.